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FACTORS ENHANCING THE RESPONSE OF THE HUMAN ADRENAL TO CORTICOTROPIN: IS THERE AN ADRENAL GROWTH FACTOR?*

GRANT W. LIDDLE, M.D.,† DONALD ISLAND, B.S., ARTHUR P. RINFRET, PH.D. AND PETER H. FORSHAM, M.D.

The Metabolic Unit for Research in Arthritis and Allied Diseases and the Department of Medicine, University of California School of Medicine, San Francisco, California, and from the Department of Chemistry, Stanford University, Palo Alto, California

INTRODUCTION

THE present study is concerned with the problem of whether there is more than one pituitary factor which exerts a direct influence on the adrenal cortex.

Pituitary corticotropin, or ACTH, as first isolated, purified, and chemically and biologically characterized by Li and his associates (1) and by Sayers and his co-workers (2) in 1943, has been amply demonstrated to possess three major functions: the maintenance of the weight and the stimulation of growth of the adrenal cortex; the discharge of ascorbic acid and cholesterol from the gland; and the synthesis and secretion of active steroids.

Evidence has recently been summarized by Li and his associates (3) regarding quantitative differences in these fundamental characteristics

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† Public Health Service Research Fellow of the National Institute of Arthritis and Metabolic Diseases. Present address: Clinic of General Medicine and Experimental Therapeutics, National Heart Institute, Bethesda 14, Maryland.

among various types of ACTH preparations, ranging from larger sized protein molecules to polypeptides obtained by partial hydrolysis.

Work by Dixon and associates (4) has indicated that certain pituitary fractions may show a marked disparity between the capacity to cause adrenal ascorbic-acid depletion and the ability to repair adrenal size and morphology in hypophysectomized rats.

In an attempt to define optimal methods for the extraction of ACTH from equine pituitaries one of the authors (A.P.R.) independently prepared pituitary fractions which would maintain, and in fact increase, the weight of adrenal glands in hypophysectomized rats, even though these same fractions were extremely poor in ascorbic-acid-depleting activity.

It is impossible to demonstrate dissociation of adrenal growth and ascorbic-acid depletion in human subjects. However clinical studies of adrenocortocotropic activity may be conducted in terms of the capacity of the adrenal cortices to secrete steroids. The rate of corticoid secretion is actually the product of two variables: 1) the "*intensity* of the ACTH *stimulus*" employed, and 2) the "*responsiveness*" of the particular adrenal cortices stimulated. These two variables may be dealt with separately. Thus by assuming the adrenocortical factor of responsiveness to be constant, it is possible to assay the steroidogenic potency of the ACTH used; and by controlling the effective dose of ACTH, it is possible to estimate the so-called responsiveness of the adrenals.

Conventional ACTH preparations stimulate the secretion of steroids by the adrenal cortex and also maintain and enhance the capacity of the adrenal to respond to subsequent stimulation. It is the purpose of the present communication to indicate that, under certain conditions, a dissociation of these two functions can be demonstrated in man. Specifically, certain pituitary fractions which possess little or no capacity to stimulate immediate secretion of adrenal steroids, nevertheless, do appear to enhance the capacity of the adrenal to respond to subsequent stimulation with ACTH.

METHODS

In order to estimate the effects of various agents upon adrenal responsiveness, subjects were given a series of standard ACTH tests during a preliminary period; the agent in question was then administered for several days; and, finally, adrenal responsiveness was reappraised by means of a second series of standard ACTH tests. In evaluating adrenal response, greatest reliance was placed upon measurements of urinary 17-hydroxycorticoids.

Subjects

Data presented here were obtained in studies of 60 adults, of both sexes, including 6 without evidence of disease, 5 with clinical adrenal insuf-

ficiency, and 8 with Cushing's syndrome; the remaining subjects suffered from various nonendocrine diseases. Studies relating to artificial enhancement of adrenal responsiveness were restricted to subjects who had shown normal initial responses to ACTH.

Techniques

Complete urine collections were made during 12-hour or 24-hour periods. A preservative for each specimen consisted of 5 ml. of a 1 per cent solution of thymol in glacial acetic acid. Urinary 17-hydroxycorticoid excretion was determined by a modification of the method of Reddy, Jenkins and Thorn (5).

Determination of total 17-hydroxycorticoids in urine

Reagents

1. Absolute butanol (any C.P. brand).
2. "Dilute sulfuric acid," in the ratio of 310 ml. of sulfuric acid to 190 ml. of water.
3. Color reagent—80 mg. of purified phenylhydrazine HCl (Eastman Reagent Grade recrystallized once from absolute ethyl alcohol) in 100 ml. of "dilute sulfuric acid" (made up fresh every other day).
4. Absolute methanol, acetone-free.
5. K_2CO_3,(10-N); and H_2SO_4,(1-N, or approximately 5 per cent)—both in dropping bottles.

Standards

Dissolve 5 mg. of cortisone or hydrocortisone or 5.57 mg. of the acetates in 50 ml. of absolute methanol as stock solution (stable for several months in the refrigerator). The stock solution (100 gamma/ml.) is diluted with methanol to give standards containing 20 gamma/ml. and 10 gamma/ml. The diluted standards are stable for approximately one week in the refrigerator.

Sample

A 24-hour urine sample is preserved by previously adding from 2 to 5 ml. of a 1 per cent solution of thymol (stored in a brown bottle) in glacial acetic acid. A 5-ml. portion of preservative is sufficient for a urine sample of up to 4,000 ml. There is no loss at room temperature for at least one week.

Butanol extraction and evaporation

Aliquots of 5 ml. or 10 ml. of urine may be used for normal subjects; a 5-ml. aliquot is best during ACTH or cortisone treatment; 20 ml. or more may be necessary in cases of adrenal insufficiency. A suitable urine aliquot is transferred to a centrifuge tube and adjusted to pH 2.0–2.5 with 5 per cent H_2SO_4 or 10-N K_2CO_3 and Hydrion paper, and extracted *at once* to prevent high blanks. Extraction at pH 2.0–2.5 rather than at pH 1 reduces the amount of extraneous pigments extracted, while relatively not affecting the amount or the ease of extraction of the Porter-Silber corticoids.

Two volumes of butanol are added to the tube, which is shaken for three minutes on a Kahn shaker (modified to shake tubes longitudinally), and spun in the centrifuge at 2,000 RPM for one or two minutes. The supernatant (butanol) layer is transferred to a

clean tube. The volume of the remaining aqueous layer is brought back to the original with H_2O, and extraction with 1 volume of butanol is carried out after readjusting the pH to 2.0–2.5. Extracts are combined. Butanol and urine samples should be separated at once to minimize the blank. Water is routinely removed from the combined extract with anhydrous Na_2SO_4 for a period not exceeding fifteen minutes. The combined butanol extracts are then shaken up with 1/10 volume of 1-N K_2CO_3 for half a minute. *Immediately* (any delay causes loss of F) sufficient anhydrous Na_2SO_4 is added to remove the aqueous base and the mixture is filtered through glass wool. In urines containing many ketone bodies, another base wash is necessary to prevent false high values. The filtrate should be clear; if not, more Na_2SO_4 is added and the material is refiltered. The base extraction removes from 25 to 45 per cent of the urinary pigment in the blank obtained at pH 2.0, without detectably affecting the corticoid recovery. An aliquot of the combined extracts (usually half or more) is evaporated to dryness in a small conical flask with a slotted cork attached to a water pump or house vacuum at 45° to 50° C. (A single water pump will evaporate a 5-ml. sample in about ten minutes.)

Colorimetry

The dried residue is dissolved in 3 ml. of methanol, and 1 ml. of the solution is placed in each of two tubes. To one of the two colorimeter tubes is added 5 ml. of "dilute sulfuric acid"; to another tube, 5 ml. of the color reagent. The cork-stoppered tubes are mixed by inversion and are then placed in a water bath at 60° ± 1° C. for twenty minutes. (Cork stoppers must be protected by Parafilm.) When removed they are cooled for three minutes and read on the Beckman DU (preferred) or Coleman (Model 14) spectrophotometer at 410 mμ. For a complete run the tubes are set up as follows:

(a) *Colorimeter blank*	(b) *Reagent blank*	(c) *Standard (10 & 20 gamma)*
1 ml. methanol	1 ml. methanol	1 ml. standard
5 ml. H_2SO_4	5 ml. color reagent	5 ml. color reagent
(d) *Standard blank*	(e) *Unknown*	(f) *Urine blank*
1 ml. standard	1 ml. extract	1 ml. extract
5 ml. H_2SO_4	5 ml. color reagent	5 ml. H_2SO_4

Sample calculation: (gammas of total compound E or F-like substances per twenty-four hours).

$$\frac{\text{R corrected unknowns (e}-\text{b)}}{\text{R corrected standard (c}-\text{b)}} - \frac{\text{R corrected urine (f}-\text{a)}}{\text{R standard blank (d}-\text{a)}} \times \frac{\text{24-hr. vol.}}{\text{urine aliq.}} \times \frac{3}{1} \times \text{butanol dilution} \times \text{standard conc. (mg.)} = \frac{\text{Total 17-OH-corticoids as mg. of E or F}}{\text{Urine volume}}.$$

Standard ACTH test

In order to gain precise control of the ACTH stimulation, use has been made of a "standard ACTH test," adhering closely to the principles outlined by Renold *et al.* (6) namely:

A. The same dose of ACTH was used in all standard tests in any one study. .

B. All standard test materials were given as a constant infusion over exactly eight hours.
C. The same "lot" of ACTH was used in all standard tests in any one study.
D. All standard test material was given by the intravenous route.
E. All standard tests were separated by two or more days without endocrine treatment.

Materials

Lyophilized ACTH, standardized by the intravenous ascorbic-acid depletion method of Sayers with potencies expressed in USP units, was used for the "standard ACTH stimulation tests." The material represented, for the most part, column-type ACTH.[1]

"Special pituitary fractions" were prepared by extraction of pituitaries with organic solvents and partial purification by fractional precipitation at varying pH's. A material designated herein as "equine anterior pituitary extract" represented a mixture of ordinary ACTH and "adrenal growth factor," in contrast to preparation EX-2b which was largely composed of the latter.[2]

Materials used in studying the effect of other pituitary factors on adrenal responsiveness consisted of somatotropic hormone and thyroid-stimulating hormone.[3]

In order to control the possible nonspecific effect of pyrogenicity on adrenal responsiveness, febrile reactions were induced deliberately by the use of Piromen,[4] a purified polysaccharide derivative of *Pseudomonas aeroginosa.*

RESULTS

Validity of the method for determining adrenocortical activity

Changes in 17-ketosteroid excretion, sodium and potassium excretion, circulating eosinophils, and blood sugar level, although useful semiquantitatively in evaluating the effectiveness of ACTH, represent indirect measurements of adrenocortical activity. In our experience the most reliable quantitative index of adrenal activity in man is the determination of urinary 17-hydroxycorticoids (Fig. 1).

We are indebted to:

[1] The Armour Laboratories, the Upjohn Company, and Parke-Davis Company for providing the ACTH employed in these studies.

[2] The Victory Packing Company of Los Angeles for a generous supply of "equine anterior pituitary extract," as well as the more highly purified special pituitary fraction, EX−2b.

[3] The Armour Laboratories for supplies of these materials.

[4] The Travenol Laboratories for generous supplies of Piromen.

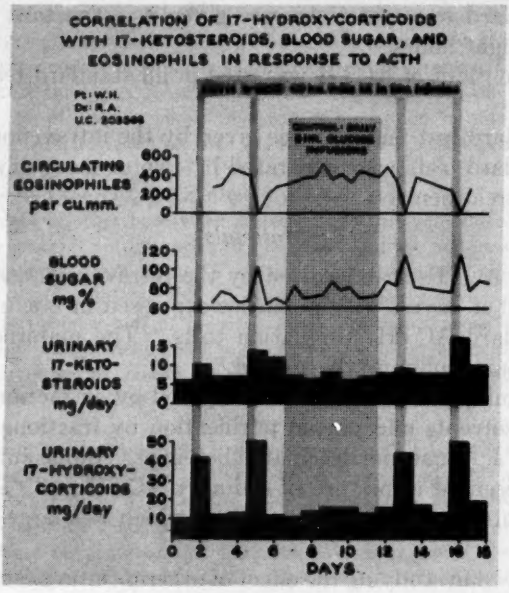
CORRELATION OF 17-HYDROXYCORTICOIDS WITH 17-KETOSTEROIDS, BLOOD SUGAR, AND EOSINOPHILS IN RESPONSE TO ACTH
CIRCULATING EOSINOPHILES per cu.mm.
BLOOD SUGAR mg %
URINARY 17-KETO-STEROIDS mg/day
URINARY 17-HYDROXY-CORTICOIDS mg/day
DAYS

FIGURE 1

The administration of either cortisone or hydrocortisone results in a rise in urinary 17-hydroxycorticoids which is directly proportional to the administered dose (Fig. 2).

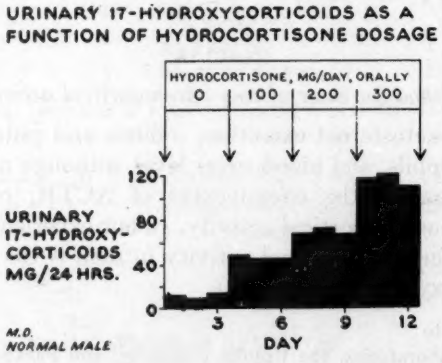

FIGURE 2

According to determinations carried out in this laboratory, the average adult excretes the colorimetric equivalent of about 10 mg. of hydrocortisone per day. The maximum range is from 5–16 mg. daily. Males as a group tend to excrete slightly more than females, although the range of values

overlaps considerably. In the absence of severe stress the corticoid excretion of patients with either acute or chronic nonendocrine disease is similar to that of normal subjects. In the presence of adrenal insufficiency, due to Addison's disease or following bilateral adrenalectomy, these values vary from 0 to 5 mg. per day. In our series of patients with Cushing's syndrome these values ranged from 15 to 70 mg. per day.

The experimental basis of the "standard ACTH test"

Since "adrenal responsiveness" cannot be employed as a quantitative concept unless one is able to control the intensity of the ACTH stimulus, it is essential that certain variables inherent in the pharmacology of ACTH be controlled.

1. Corticoid secretion is in part a function of the *dose of ACTH*. As illustrated in Figure 3, the logarithm of the dose of ACTH bears a rectilinear relationship to the resulting increase in excretion of urinary 17-hydroxycorticoids over a considerable dose range. If one is to judge changes in

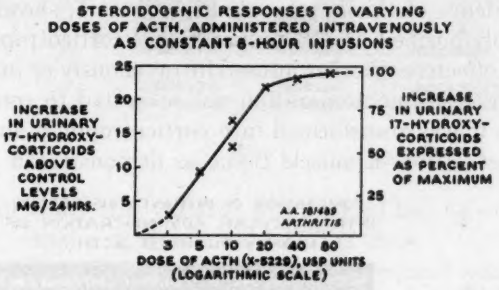

FIGURE 3

adrenal responsiveness by means of a series of "ACTH tests" it is obviously necessary to employ the same dose of ACTH in carrying out all standard tests in any one study. Furthermore, since the unitage of ACTH is based upon a bioassay procedure which may give results varying as much as 20 per cent (7), it is necessary that the same "lot" of ACTH be employed in all standard tests in any one study.

2. If the dose of ACTH be kept constant, and the *duration of administration* be varied, it is found that the same dose of ACTH acts as a more potent stimulus if administered over a longer period of time (Fig. 4). For this reason it is necessary to conduct all standard test infusions over exactly the same period of time, if one is to obtain valid information with which to compare the adrenal responsiveness on various occasions.

3. When ACTH is injected intramuscularly or subcutaneously, a portion of it is inactivated before it reaches the adrenals. The degree of inactivation

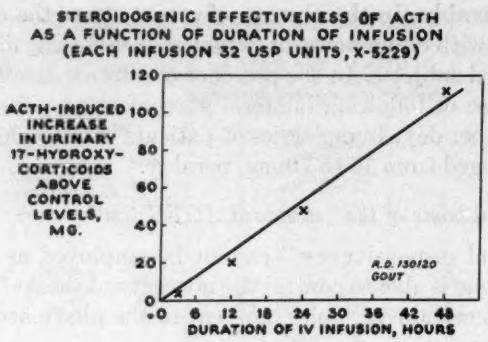

FIGURE 4

varies from one preparation of ACTH to another and from one subject to another. This susceptibility of ACTH to inactivation apparently depends to a considerable degree upon the chemical nature of the ACTH preparation itself. Evidence of this is presented in Figure 5, showing that 8 USP units of an highly purified ACTH preparation[5] "corticotropin A" (8), was almost equally effective whether infused intravenously or intramuscularly. However, when this same preparation was subjected to controlled partial acid hydrolysis it was transformed into corticotropin B and became susceptible to inactivation in muscle tissue as demonstrated by the finding

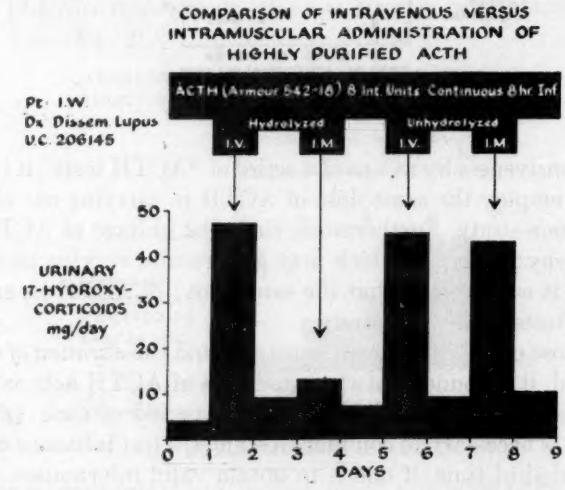

FIGURE 5

[5] These materials were prepared by Mr. J. D. Fisher of Armour Laboratories, Chicago.

that when 8 USP units of this product was infused intramuscularly, its steroidogenic activity was minimal even though it retained its original potency when given by the intravenous route.

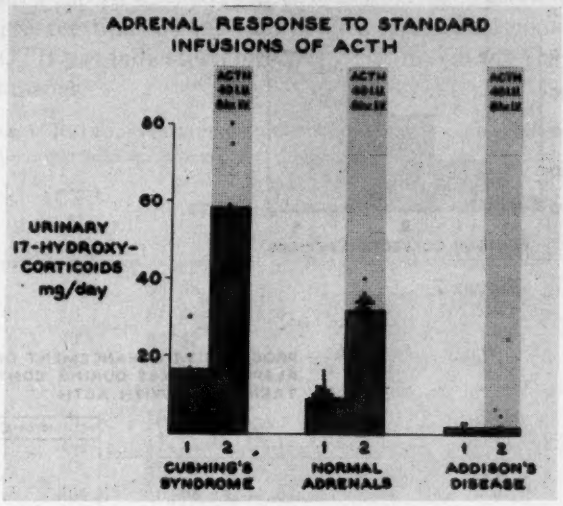

FIGURE 6

In the present study this variable was avoided by the use of the intravenous route in conducting all standard ACTH tests.

4. As illustrated in Figures 7, 8, 9 and 15, frequent administration of ACTH results in a progressive enhancement of adrenal responsiveness. For this reason it is necessary to separate "standard ACTH tests" by adequate periods without stimulation, in order to obtain reproducibility of adrenal secretory responses.

Reproducibility of responses to standard ACTH tests

When an identical dose of the same ACTH preparation is administered to the same subject as a constant intravenous infusion over an eight-hour period on intermittent days, using the same time of day in order to reduce changes due to diurnal variation in adrenocortical activity, the adrenal response in terms of urinary 17-hydroxycorticoids is reproducible[6] (Figs. 1, 9, 10, 11 and 12). In order to evaluate the reproducibility of such re-

[6] As a rule, if ACTH tests are repeated at intervals of less than four days, uniform reproducibility of adrenal responses is not established until after the first such test, which in a sense serves as a "priming" adrenal stimulus. For this reason the initial ACTH test has been neglected in presenting these studies, with the exception of Figures 6 and 15.

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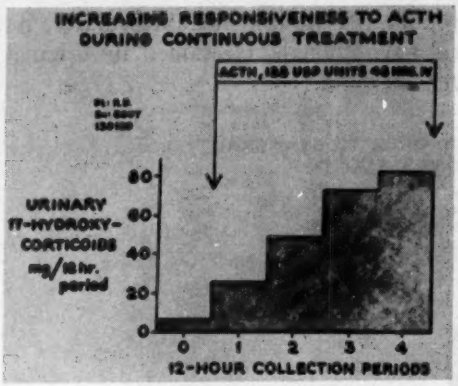

FIGURE 7

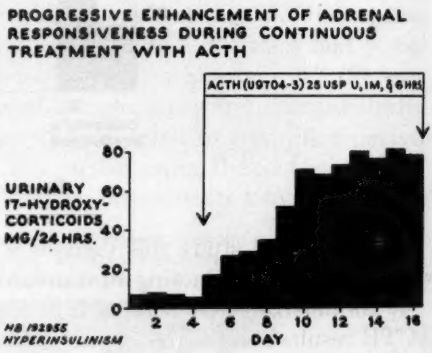

FIGURE 8

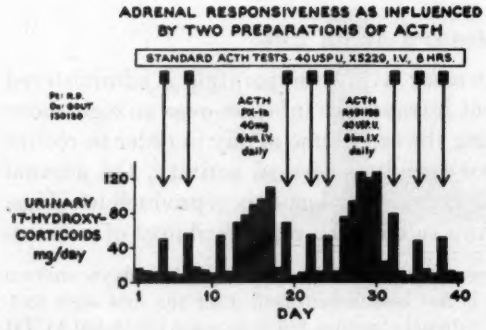

FIGURE 9

sponses in any one individual, 12 subjects were given a total of 69 standard ACTH tests and the results were tabulated (Table 1). The greatest deviation of any response from the mean of its respective series was 25 per cent. We have taken the view, therefore, that for any one individual to show a change of greater than 50 per cent in his adrenal responsiveness to a standard ACTH test indicates a high degree of probability that this change is not spontaneous.

TABLE 1. REPRODUCIBILITY OF RESPONSES TO STANDARD ACTH TESTS

Subject	ACTH (units/8 hrs.)	Urinary 17-hydroxycorticoids (mg./24 hrs.)	Mean	Maximum deviation
He.	10	58, 68, 64	63	−8%
Scr.	10	31, 33, 29, 34, 31	32	−10%
We.	40	60, 47, 55	54	−13%
Do.	40	36, 40, 37, 42, 43, 44, 51, 51, 52 48, 57, 51, 48	46	+24%
Do.	10	15, 15, 15	15	0
Li.	25	11, 11, 11, 12, 11, 11	11	+9%
Al.	40	31, 30, 32	31	±3%
Ha.	40	46, 50, 44, 54, 64	52	+23%
Pe.	40	42, 45, 40	42	+7%
Pe.	10	35, 38, 44, 35, 32, 38	37	+19%
Wi.	40	37, 48, 46, 58, 56, 51, 50	49	−25%
Ar.	10	41, 45, 41	42	+ 7%
Ki.	40	53, 50, 60	54	+11%
Sca.	25	33, 38, 32, 35, 28, 40	34	±18%

Pathologic alterations of adrenal responsiveness

As noted previously, the normal range of values of 17-hydroxycorticoid excretion is fairly well defined, but there may be some overlap of individual values obtained in patients with Cushing's syndrome and in normal subjects on the one hand, and patients with Addison's disease and normal subjects on the other hand. However, the difference between groups becomes distinct during standardized stimulation with ACTH (9, 10). Thus, when subjects without adrenal disease are *initially* treated with 25 (or more) international units of ACTH given as a constant infusion over an eight-hour period, 17-hydroxycorticoid excretion rises to 20–40 mg. during the corresponding day. Similar standard ACTH tests given to a series of patients with Cushing's syndrome resulted in corticoid excretions ranging from 50 to 90 mg. per day (Fig. 6). On the other hand, standard ACTH tests given to a series of patients with Addison's disease resulted in corticoid excretions of from 0 to 6 mg. per day. Clearly, then, there are naturally

occurring alterations in adrenal responsiveness, and it can be demonstrated that these alterations may be simulated under experimental conditions.

Artificial enhancement of adrenal responsiveness

ACTH. It is possible to induce an increase in adrenal responsiveness by continuous treatment with ACTH. This principle is illustrated in Figure 7, which shows the twelve-hourly corticoid excretion of Subject R.D. during a 48-hour continuous intravenous infusion of a total dose of 128 USP units of ACTH. Since the intensity of the ACTH stimulus was identical during each twelve-hour period, it is apparent that this subject's adrenals were considerably more responsive to ACTH during the final period than they were during the first period. The same general pattern is apparent in subjects treated on successive days with ACTH given according to a schedule which permits the ACTH to act in significant doses over several hours on each day. Such schedules include the administration of ACTH as an intravenous infusion for eight or more hours daily, the intramuscular injection of an aqueous suspension of ACTH every six hours (Fig. 8), or the intramuscular injection of ACTH in 16 per cent gelatin every twelve to twenty-four hours. That this is not due to delayed appearance of corticoids in the urine is shown by the fact that corticoid excretion is near or at the pretest level on the day following individual tests (Figs. 9 and 10). The in-

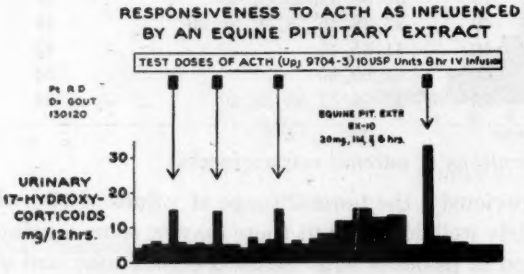

FIGURE 10

crease in adrenal responsiveness, which is induced by continuous treatment with ordinary ACTH, is not usually of more than a few days' duration. We have studied adrenal responsiveness to standard ACTH tests before, and again following, intensive courses of treatment with several different ACTH preparations. Typical studies are represented in Figure 9. During the initial period of testing, adrenal responsiveness was fairly uniform. The administration of ACTH for five successive days was accompanied by a stepwise increase in 17-hydroxycorticoid excretion. When standard testing was resumed, adrenal responsiveness was found to be

slightly greater than before, but it then rapidly returned to pretreatment levels. A second course of treatment with a different preparation of ACTH gave much the same result.

Miscellaneous pituitary fractions

Several fractions of *equine pituitary extracts*, prepared by A.P.R., appeared to produce an increase in adrenal responsiveness which was out of proportion to their immediate steroid-discharging activity. One of these studies is illustrated in Figure 10. This raised the question as to whether enhancement of adrenal responsiveness could actually be brought about by preparations which lacked completely the immediate steroid-discharging activity of ACTH. Of the preparations which have been available to us, only one ("EX-2b") seemed to show complete dissociation of these effects in man. In the study illustrated in Figure 11, preliminary testing with standard infusions of ACTH showed satisfactory uniformity of adrenal responsiveness. In contrast to previous studies (Figs. 3–10), the intravenous infusion of this special fraction for eight hours on each of five successive

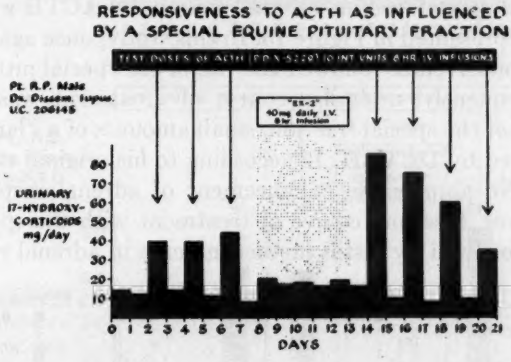

FIGURE 11

days was accompanied by practically no immediate increase in corticoid excretion. This we have interpreted as evidence that there was practically no conventional ACTH in this preparation. However, when standard ACTH tests were resumed, a rather marked increase in adrenal responsiveness was seen, which gradually waned over several days.

The finding to be emphasized is not merely that an increase in adrenal responsiveness was induced, but rather that it was induced, apparently, by a preparation which had little or no immediate steroid-discharging activity of its own. Figure 12 represents a study in another subject using the same material and showing much the same result. Repeated use of the standard ACTH test was associated with no remarkable change in adrenal

responsiveness until after the course of treatment with the special pituitary fraction, when a rather marked increase in responsiveness was seen.

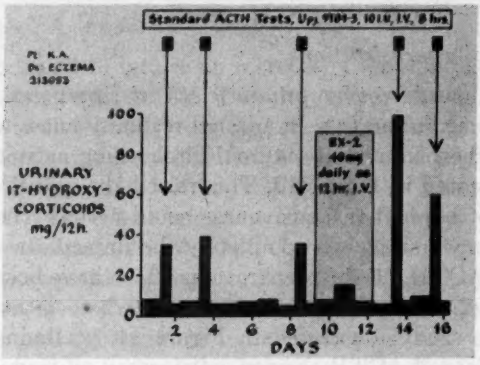

FIGURE 12

The question as to whether this whole pattern could be produced by the presence in this special fraction of small amounts of ACTH was approached in the study represented in Figure 13. In this study, once again, an increase in adrenal resopnsiveness followed the use of the special pituitary fraction EX-2b. Subsequently, a similar course of treatment was administered using, instead of the special fraction, small amounts of a "large molecular" ACTH prepared by Dr. C. H. Li according to his original method of purification (1). No appreciable enhancement of adrenal responsiveness resulted. However, a second course of treatment with the special pituitary fraction was followed by a still further increase in adrenal responsiveness.

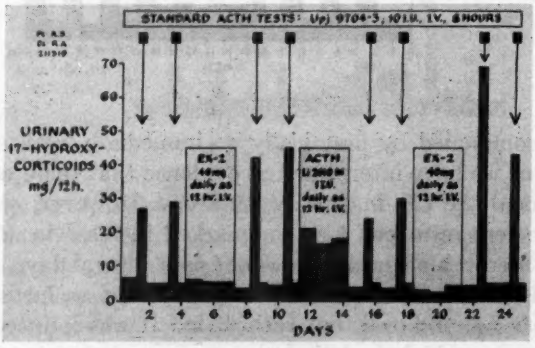

FIGURE 13

Since this special pituitary fraction has not as yet been well characterized chemically or biologically,[7] the question arose as to the specificity of this

effect on adrenal responsiveness. The following studies bear on this point.

Somatotropic hormone. As illustrated in Figure 14, the use of a highly purified somatotropic hormone preparation in doses of 75 mg. daily for five successive days had no perceptible influence on adrenal responsiveness in 2 subjects.

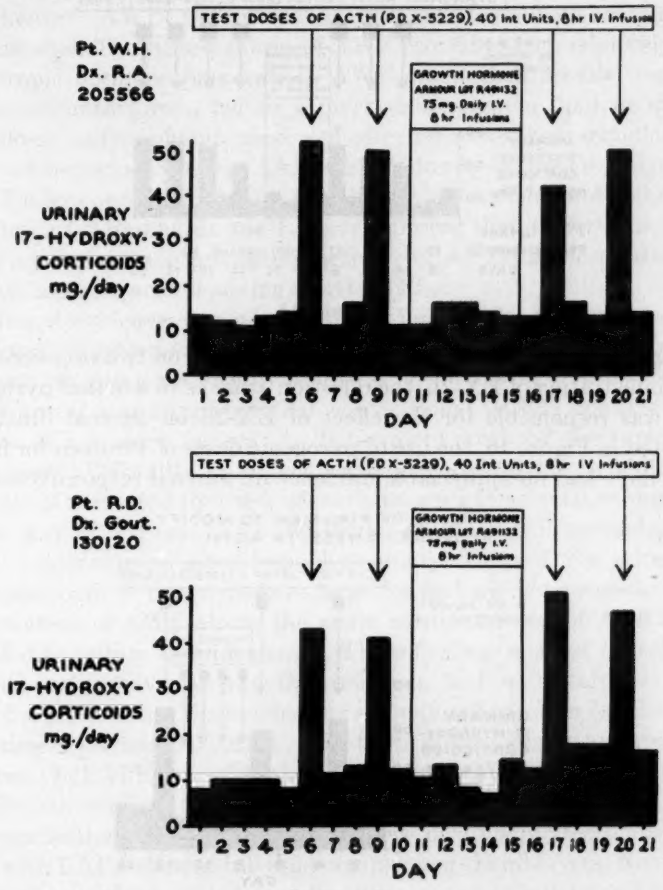

FIGURE 14

[7] Biologic assays in rats and guinea pigs indicate that this preparation contains no detectable quantities of thyroid-stimulating hormone or gonadotropic hormones. Adrenal weight of the hypophysectomized rat is partially maintained by the subcutaneous injection of 1.0 mg. of this special fraction daily for twenty-one days. In terms of ascorbic-acid depletion, 40 mg. of the special fraction is estimated to contain no more than 1 USP unit of ACTH.

Thyroid stimulating hormone. As illustrated in Figure 15, the use of an active thyroid-stimulating hormone in doses of 30 USP units daily for four days had no very marked effect on adrenal responsiveness.

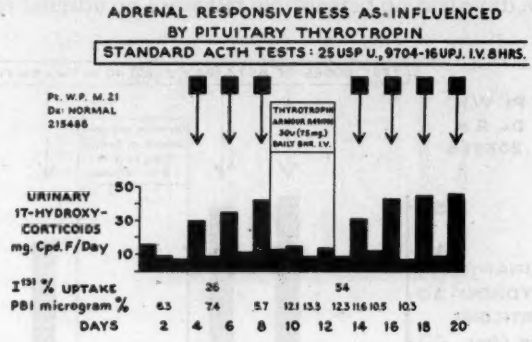

FIGURE 15

Pyrogens. Since febrile responses were observed on two occasions during the administration of EX-2b, the question arose as to whether pyrogenicity *per se* was responsible for the effect of EX-2b on adrenal function. As illustrated in Figure 16, the use of pyrogenic doses of Piromen for four successive days had no appreciable influence on adrenal responsiveness.

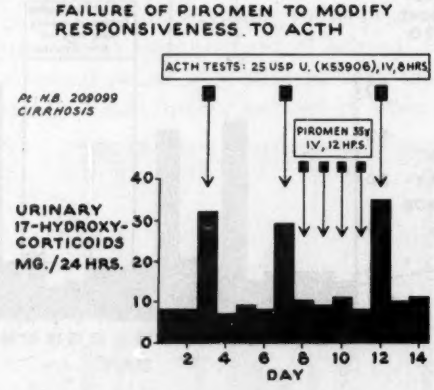

FIGURE 16

DISCUSSION

The question as to whether the adrenal cortex is subject to more than one type of influence arising from the pituitary has stimulated controversy for over a decade. For example, it has been suggested by Albright and others that the pituitary gonadotropins modify the qualitative pattern of

adrenal steroid production (11). It has also been reported by Dixon and associates (4) that one might obtain from pituitary extract fractions which are extremely potent as adrenal weight factors, but which are poor in the adrenal ascorbic-acid depleting activity so characteristic of conventional ACTH. Both the "adrenal ascorbic-acid factor" (AAF) and the "adrenal weight factor" (AWF) were prepared in impure form from acetone-dried hog pituitaries. The richest source of AWF proved to be a relatively crude somatotropic hormone preparation. AWF maintained adrenal weight in hypophysectomized rats, led to a marked increase in lipid in the subglomerulosal sudanophobic zone and effected a marked stimulation of mitosis in this region; whereas AAF failed to do any of these to a significant degree. Under conditions of acid hydrolysis AWF seemed more labile than AAF. Similarly, Reinhardt and Li have reported that under acid hydrolysis the adrenal growth-promoting activity of ACTH is more labile than is adrenal ascorbic-acid depleting activity (12).

Additional evidence was presented by Young's laboratory to suggest that adrenal weight-maintaining activity may also be separated from ascorbic-acid depleting activity by a process not involving hydrolysis. From these results it was suggested that conventional ACTH consisted of at least two, if not more factors (4). Subsequent work by the same group showed that AWF failed to induce steroidogenesis in the hypophysectomized rat, as measured by titers of corticosterone in adrenal venous blood (13). By way of contrast, AAF did induce an increase in steroidogenesis, if given continuously, after hypophysectomy. Even if the adrenals of hypophysectomized rats were kept large for as long as two weeks by the administration of AWF alone, the acute administration of AAF at that time failed to induce steroidogenesis. These findings suggest that AWF is concerned exclusively with growth promotion, AAF with steroid synthesis and discharge, and that the two factors are required together for the proper functioning of the adrenal cortex. The effects of the "equine anterior pituitary extract" (EAPE) on adrenal structure proved identical to that found by the British workers with their "adrenal weight factor" (AWF). Histologic examination of the adrenal cortices of hypophysectomized rats treated with EAPE showed full lipid complements in all layers. No sudanophobic zone was observed between the outer zona fasciculata and the zona glomerulosa of EAPE-treated rats, in contrast to rats receiving conventional ACTH over a wide dosage range. Nonhypophysectomized rats receiving EAPE likewise exhibited intensely staining sudanophilic material throughout all layers of the thickened adrenal cortices.

Somatotropic hormone, prepared by the method of Wilhelmi *et al.* (14), was used in similar experiments but was found to be inactive in maintaining adrenocortical morphology following hypophysectomy.

Unfortunately, some of the studies which have been conducted in animals showing a dissociation of adrenocorticotropic effects have employed the intravenous route of administration in testing for ascorbic-acid depleting potency and some extravascular route in testing for adrenal growth-promoting activity. As emphasized by Thompson and Fisher (15), proof that ascorbic-acid depleting activity and adrenal weight-maintaining activity are separable requires that the same route of administration be employed in testing for each activity. Thus, a preparation which shows good potency in depleting adrenal ascorbic acid when administered *intravenously* may show little potency in maintaining adrenal weight when administered *subcutaneously*. Such a discrepancy might merely be due to the fact that the preparation in question is highly susceptible to inactivation by tissues at the site of injection.

Part of the significance of the present clinical study rests with the fact that the intravenous route of administration of all agents was consistently employed. On the basis of the present study, it appears possible to bring about an increase in the responsiveness of the adrenal cortex to standard ACTH stimulation not only by administering ACTH itself but also by the intravenous infusion of a pituitary fraction which lacks the immediate steroid-discharging property of ACTH.

It need not be assumed that the anatomic bulk of a gland is necessarily related to the functional activity of that gland, whether measured in terms of basal secretion or in terms of responsiveness to exogenous stimulation. Whether the so-called "*functional growth*" of the adrenal cortex produced by this special pituitary fraction has a counterpart in *anatomic growth* of the adrenals is not answered by the present clinical studies. Whether the effects observed with the use of this particular unidentified pituitary fraction represent the action of a distinct hormone, or whether the differences which occur between the special fraction and highly purified ACTH are artifacts appearing during the fractionation of pituitary extracts, remains to be determined.

If it be true that the pituitary influence upon the adrenal cortex is of a dual nature, *viz.*, trophic and secretory, effected by distinct pituitary factors, then this relationship would appear to be analogous to the hypothalamo-hypophyseal-thyroid (16) relationship on the one hand and the pituitary-gonad (17) relationship on the other. It has been reported that, in each system, the action of the pituitary in maintaining the "target" glands morphologically may be differentiated from the action of the pituitary in stimulating those glands to actual secretory activity. Bearing directly on this point is the work of Hume (18), showing that destruction of the median eminence of the hypothalamus results in a full-sized adrenal which is unresponsive to stress.

SUMMARY

The determination of urinary 17-hydroxycorticoids together with the use of a "standard ACTH test" has been applied in the quantitative estimation of adrenal "responsiveness." Adrenal responsiveness is above normal in patients with Cushing's syndrome, and is distinctly subnormal in patients with either primary or secondary adrenal insufficiency. An increase in adrenal responsiveness may be demonstrated not only during the continuous administration of ACTH, but also during treatment with a special pituitary fraction which is devoid of the immediate steroid-discharging property of ACTH. Whether this special fraction represents a distinct hormone or an artifact produced during the purification of pituitary extracts remains to be determined.

Acknowledgments

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EFFECTS OF X-IRRADIATION ON THE HYPOTHALAMUS:

A POSSIBLE EXPLANATION FOR THE THERAPEUTIC BENEFITS FOLLOWING X-IRRADIATION OF THE HYPOPHYSIAL REGION FOR PITUITARY DYSFUNCTION*

ARTHUR ARNOLD, M.D.

The Department of Neurology and Neurological Surgery, at the Neuropsychiatric Institute, University of Illinois College of Medicine, Chicago Illinois

INTRODUCTION

THE primary purpose of this report is to direct attention to certain histologic changes observed in the nuclear masses of the hypothalamus of the monkey, following x-irradiation, which may explain in part some of the therapeutic benefits obtained by x-irradiation of the hypophysial region for a number of clinical disorders thought to be due to pituitary dysfunction. For the past four years studies have been in progress to determine and analyze the effects of high energy x-rays on the central nervous system of primates (1, 2), and during the course of such studies it was noted that the paraventricular and supraoptic nuclei of the hypothalamus were rather responsive to irradiation. Since the normal hypophysis has been frequently reported as being radio-resistant and, in view of the increasing evidence that the hypothalamus (3–6) mediates or controls many of the functions of the pituitary body, one may then postulate that the therapeutic benefits reported to follow x-irradiation of the hypophysial region for a variety of clinical disorders, may be due, in part, to the effects of x-irradiation on the nuclear structures of the hypothalamus. It should be emphasized that these observations were made on the monkey.

REVIEW OF THE LITERATURE

For many years radiation therapy has been administered to the region of the hypophysis for the treatment of disorders related to pituitary dysfunction, such as malignant exophthalmos (7), selected cases of hypertension (8), and various gynecologic conditions of functional origin, such as some cases of amenorrhea, oligomenorrhea and sterility (9–11). The clinical results obtained with x-ray irradiation of the hypophysial region

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in such cases of pituitary or hypothalamic dysfunction, have been gratifying but the explanation for the clinical successes has never been fully clarified. As a matter of fact, several investigators (12–14) have indicated that the hypophysis is resistant to high doses of x-irradiation. Fehr (14), for example, administered single doses of 2,360–2,645 r and short fractionated courses totaling 3,300–10,160 r of 180 kv. x-rays to the pituitary region of sexually mature rabbits and was unable to detect any histologic changes in the hypophysis of animals sacrificed from four to six weeks after radiation. Fehr, therefore, concluded that the "pituitary was radio-resistant." However, Kotz *et al.* (15), although unable to detect any histologic alteration in the hypophysis of their irradiated animals, wisely cautioned that the absence of anatomic changes could not be interpreted as indicating a lack of functional change. Lawrence *et al.* (16), in an extensive study of the effects of 180 kv. x-rays on the pituitary gland of the albino rat, concluded from their observations that the pituitary gland of the albino rat was relatively radio-resistant but that the glands could be influenced by x-rays. They noted after irradiation of the pituitary region of 30–40 day old female albino rats, a temporary decrease in the growth rate of the rats, followed by a gradual recovery. These workers adhered to two dose-schedules, as follows: 1) 513 r at each exposure, five days apart for three or four times, and 2) two doses, each of 1,040 r, with short time intervals between.

In general, the literature indicates that even though the pituitary body has been labeled radio-resistant, on the basis of histologic studies, pituitary function may be altered by x-irradiation of the region of the hypophysis. No report could be found in the literature pertaining to a careful study of the hypothalamus following x-irradiation of the pituitary region. In irradiating the pituitary region the ventral portions of the hypothalamus are usually unavoidably irradiated as well.

EXPERIMENTAL METHOD

These studies, as previously indicated, are part of a comprehensive study to determine and analyze the effects of high energy x-rays on the central nervous system of primates, *i.e.*, the monkey (*Macaca mulatta*) and man (1, 2). The source of x-rays is the medical betatron at the University of Illinois, which yields high energy x-rays at 23 million volts (23 mev.).

When compared to x-rays obtained from conventional x-ray machines at energies of 200–400 thousand volts (200–400 kv.), high energy x-rays at 23 mev. are approximately one hundred times more energetic. This high energy potential of the betatron enables one to produce beams of x-rays which are 1) free from any significant side-scatter, 2) more penetrating, and 3) more homogeneous in dose distribution than x-ray beams of low energy. It should be indicated at this time that the physical dose unit (r) as pro-

duced by the betatron is approximately 40 per cent less effective, biologically, than the r as produced by the conventional x-ray machine of low energy. The betatron r is the reading of a 25 r Victoreen thimble chamber surrounded by sufficient material for electron equilibrium. This decreased effectiveness of high energy x-rays has been demonstrated in a number of studies (17–20). For example, a dose of 3,000 r of 23 mev. x-rays is biologically equivalent to a dose of 1,800 r of 400 kv. x-rays. Therefore, throughout this paper, whenever a dose of high energy x-rays is given, its equivalent dose of low energy x-rays will also be given.

In the actual experimental exposure small beams of high energy x-rays were fired through the brains of healthy adult monkeys (*Macaca mulatta*) of both sexes, weighing 2.5 to 4.0 kilograms. Each monkey was given, while under Nembutal anesthesia, its total dose of x-rays in a single exposure to 1.0-cm. or 2.5-cm. circular beams of x-rays. The radiation was directed transtemporally or transfrontally. The monkey's head was held horizontally by means of a specially devised holder so that the entry portal of the beam was in the right temporal or frontal region, and the exit portal was in the left temporal or frontal region, respectively. The dose rate of irradiation with the 1.0-cm. beam was 150 r/min.; the dose-rate with the 2.5-cm. beam was 75 r/min. No filter was necessary with the 1.0-cm. beam, whereas a compensating filter was employed with the 2.5-cm. beam. In all cases the target-skin distance was 83.4 cm.

To date 65 monkeys have been exposed to single doses of 23 mev. x-rays from 375 r to 14,000 r. This dose range is equivalent to 225 r–8,400 r of 400 kv. x-rays. Figure 1 shows the appearance of the dose-distribution curves of 400 kv. x-rays and 23 mev.

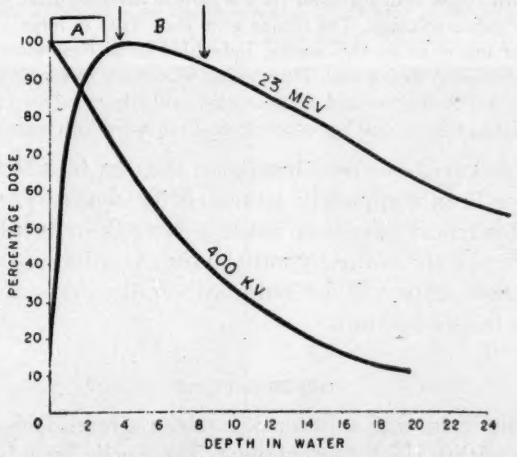

FIG. 1. Depth-dose distribution of 23 mev. and 400 kv. x-rays, as measured in cm. of depth in water. **A** represents a block of lucite, 3.4 cm. in thickness, employed to absorb the ascending portion of the dose-distribution curve of 23 mev. x-rays. **B** represents position of monkey's head in the dose curve.

x-rays, as measured in water. It will be noted that with 400 kv. x-rays the maximum dose (maximum ionization density) occurs immediately near the surface, and thereafter the dose decreases exponentially with increasing depth. On the other hand, 23 mev. x-rays produce their maximum dose at a depth of 4 cm. in water. Inasmuch as the tissues

of the body have a very high content of water and, since the atomic number of tissues is only slightly higher than that of water, the maximum dose of 23 mev. x-rays in tissues is also at an approximate depth of 4.0 cm. A 3.4-cm. block of lucite was placed against the right side of the monkey's head in order to increase the intensity of ionization on the head surface to about the 100 per cent peak of the dose-distribution curve of 23 mev. x-rays. Since the dose-distribution curves of the 1.0-cm. and 2.5-cm. beams of 23 mev. x-rays have a nearly flat plateau of between 90–100 per cent ionization density for about 4.5 cm. of tissue, and since the average transfrontal and transtemporal diameters were 5 cm. in the monkeys selected for radiation, it was possible to maintain an almost uniform tissue dose distribution through the monkey's brain. In Figure 1, the rectangle labelled A represents the block of lucite, 3.4 cm. in thickness, which was employed to absorb the ascending portion of the dose-distribution curve of 23 mev. x-rays, and the two arrows indicate the position of the monkey's head (B) in the dose-distribution curve. Thus, by this experimental procedure, it was possible to secure an almost uniform dose distribution through the monkey's brain and to detect bilateral areas of comparable radio-responsiveness within the brain.

Following the administration of the selected total dose of radiation at a single exposure, each animal was carefully observed for any clinical evidence of radiation damage. Monkeys were then sacrificed at selected intervals for histologic study. Some of the monkeys have been observed over a period of two years, or longer, particularly those receiving the lower dosages of x-rays, but their brains have not been completely studied as yet. The animals were sacrificed with intravenous Nembutal, and the intact brains were perfused with 10 per cent formalin via the carotid arteries, after preliminary washing with isotonic saline solution. The brains were then fixed in large volumes of 10 per cent formalin for ten days to two weeks, imbedded in nitrocellulose, and the entire brain serially sectioned at 20 microns. Three series were made, beginning with the twentieth, twenty-first and twenty-second sections and every twentieth section saved thereafter. Nissl, Weil-van Gieson and hematoxylin-eosin preparations were made.

Sixty-five monkeys have been irradiated thus far for all our studies and 40 brains have been completely sectioned for study. The observations pertinent to this report have been made in 6 monkeys in which the radiation had traversed the entire hypothalamus. As additional monkeys are sacrificed, further data will be obtained on the responsiveness of the hypothalamus to x-irradiation.

OBSERVATIONS

The paraventricular and supraoptic nuclei are readily identifiable cellular aggregates within the hypothalamus. Their cells have been known for some time to be concerned with the conservation and distribution of body water (3), and more recently perhaps with the partial control of the thyrotropic (6) as well as the adrenotropic hormones (5). Figure 2 shows the healthy appearance of the paraventricular nuclei, as observed in the nonirradiated hypothalamus of an adult monkey. By contrast Figure 3 reveals the appearance of the paraventricular nuclei in a monkey sacrificed at six months following the administration of 3,000 r of 23 mev. x-rays, given in

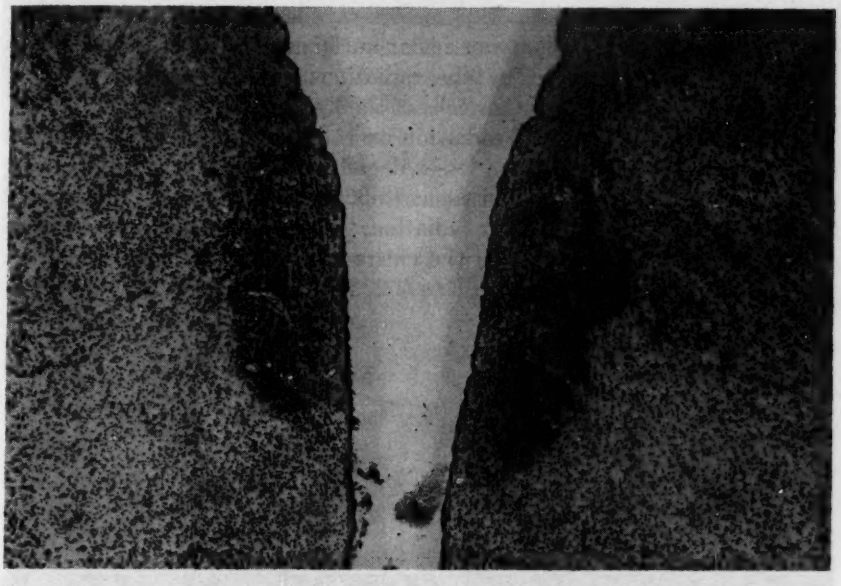

FIG. 2. The normal appearance of the paraventricular nuclei of the hypothalamus in an adult monkey. Thionin stain. ×45.

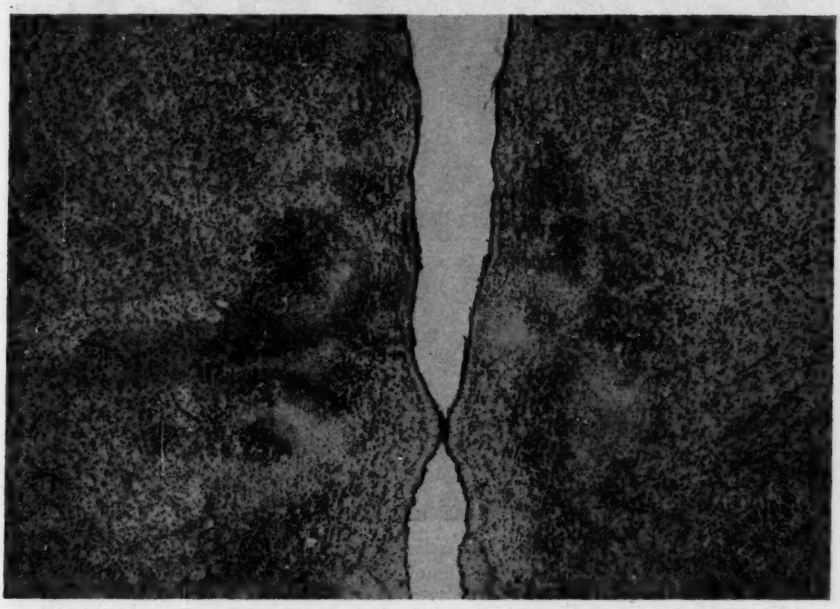

FIG. 3. The appearance of the selective damage of the paraventricular nuclei by x-irradiation. Thionin stain. ×45.

a single exposure. This dose of 23 mev. x-rays is equivalent to 1,800 r of 200–400 kv. x-rays. The paraventricular nuclei and supraoptic nuclei were almost selectively affected by the radiations, whereas only very mild changes were noted in the other cells of the hypothalamus.

This selective effect of x-irradiation on the paraventricular and supraoptic nuclei was observed to a lesser degree in a monkey which received 1,500 r of 23 mev. x-rays (equivalent to 900 r of 200-400 kv. x-rays) and was sacrificed five months after radiation. To date, 6 monkeys[1] have been studied histologically, in which the entire hypothalamus was irradiated. The actual hypothalamic tissue doses of 23 mev. x-rays for these 6 monkeys were:

1. 1,500 r—1 monkey
2. 3,000 r—2 monkeys
3. 5,000 r—2 monkeys
4. 7,000 r—1 monkey

With dosages of 23 mev. x-rays above 3,000 r the entire hypothalamus is usually seriously damaged and the cortical areas through which the radiation passes are affected as well.

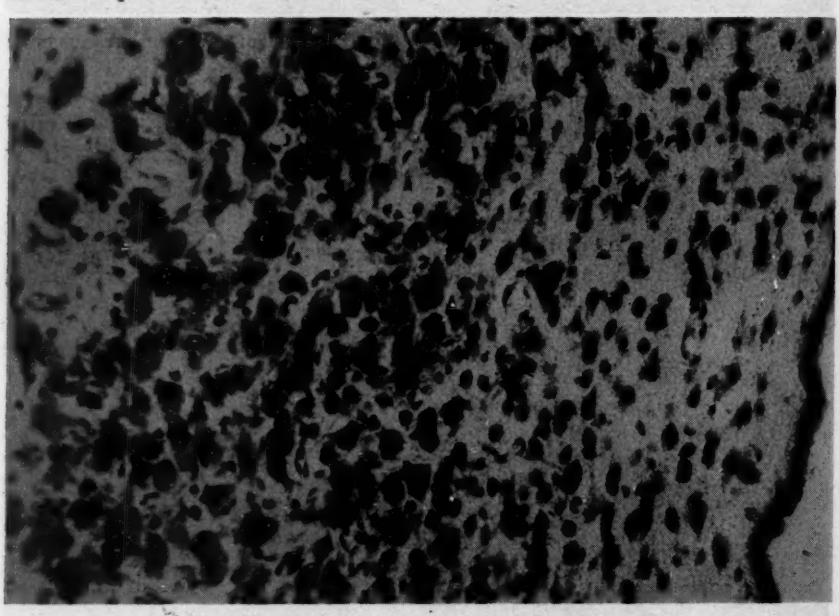

FIG. 4. The normal appearance of the cells of the paraventricular nucleus in an adult monkey. Thionin stain. ×300.

[1] Note added in proof: Five additional monkeys have shown comparable hypothalamic changes.

The mechanism for this somewhat selective effect of x-rays upon the paraventricular and supraoptic nuclei has not been determined. It would appear to be a degenerative effect resulting from x-irradiation. Figure 4 demonstrates the appearance of the cells of the normal paraventricular nucleus. Figure 5 shows the severe changes in the cells as a late effect of irradiation. The normal healthy cells of both the paraventricular and supraoptic nuclei have a rather clear, oval to rounded nucleus with a prominent, heavily staining nucleolus. They possess very prominent

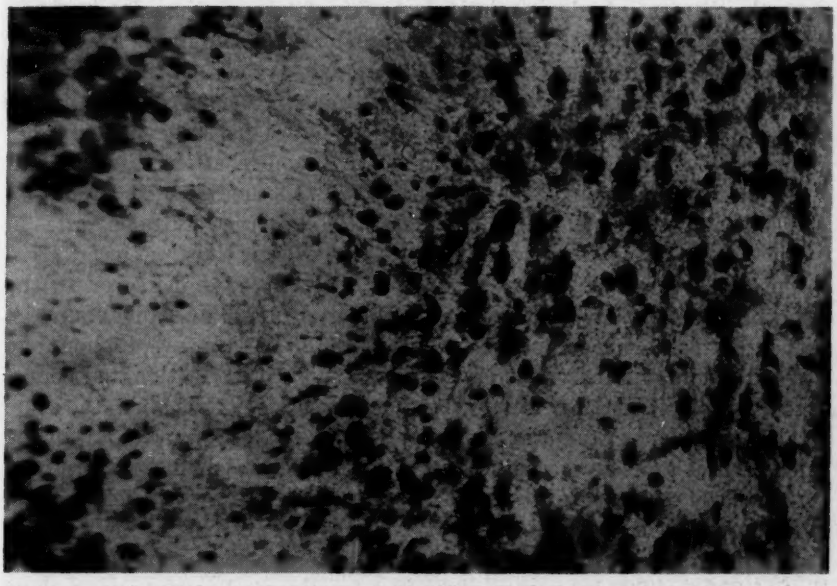

FIG. 5. The appearance of the cells of the irradiated paraventricular nucleus. Thionin stain ×300.

Nissl granules within their cytoplasm; these have an unusual distribution when compared with the neurons of the cortex, in that the Nissl material is distributed peripherally against the cell wall and hence gives the appearance of a neuron undergoing acute chromatolysis. This is, however, a normal condition for these cells. On the other hand the irradiated hypothalamic nuclei, when examined from five to six months after irradiation, show a considerable deficit of cells and all the surviving cells show degenerative changes. Many of the cells at this time have an irregular outline or are completely abnormal in shape. Some of the cells have lost all their normal cytoplasm and appear as bare nuclei. There is very little or no reactive gliosis about the affected areas. This defect in the ability of the glial cells to

participate in reactive gliosis following irradiation has been a constant observation in our studies.

DISCUSSION

The observation that the nuclear masses of the hypothalamus can show degenerative changes following x-irradiation with moderate doses of x-rays may explain in part the clinical effects observed when the pituitary region is irradiated for a number of disorders related to pituitary dysfunction, particularly in view of the reports in the literature which tend to indicate that the pituitary body is relatively resistant to radiation. It should be emphasized that these observations were made in monkeys five to six months after irradiation. Interestingly enough, Beierwaltes (7) noted, in treating patients with malignant exophthalmos by means of x-irradiation directed to the region of the hypophysis, that his patients showed a beginning recession of their exophthalmos three months after radiation, and a maximum recession at an average period of 11.8 months after radiation. These delayed time-intervals after irradiation, observed by Beierwaltes in in his clinical study, conform rather strikingly with the time-intervals in the present study. The computed tissue dose of x-rays delivered to the hypothalamus by Beierwaltes was approximately 1,800 r of 200 kv. x-rays. Although this dose is comparable to 3,000 r of 23 mev. x-rays, it should be pointed out that Beierwaltes gave his total dose in a fractionated course over a period of ten days, which would have less effect than when the same total dose is administered in a single exposure. On the other hand, in the experimental animals of the present study moderate changes were noted in the cells of the nuclei of the hypothalamus with 1,500 r of 23 mev. x-rays, which is equivalent to 900 r of 200 kv. x-rays. This dose of high energy x-rays given in one exposure may be equal to, or slightly more effective than, 1,800 r of 200 kv. x-rays given in a fractionated course over a short interval of ten days.

However, the author does not desire to enter into a discussion of radiation dosimetry but wishes rather to indicate that the hypothalamus is responsive to x-irradiation and that alterations in the paraventricular and supraoptic nuclei could in turn affect the neural control of the hypophysis and its functions. Studies are now in progress to determine whether histologic changes can occur in the hypophysis at late intervals after irradiation, since the literature fails to reveal any long-term studies of the effect of x-rays on the pituitary body; it may be that delayed histologic changes can occur in the pituitary body following relatively small dosages of x-rays. Should such changes occur, then both the hypothalamus and the hypophysis would contribute to an explanation of the therapeutic benefits following x-irradiation of the hypophysial region for pituitary dysfunction.

SUMMARY

1. The paraventricular and supraoptic nuclei of the hypothalamus are responsive to x-irradiation.

2. Since the hypothalamus, in particular the paraventricular and supraoptic nuclei, exerts a considerable influence over the function of the pituitary body, and since the pituitary body is reported to be relatively radioresistant, then perhaps the therapeutic benefits reported following x-irradiation of the hypophysial region for pituitary dysfunction are due to the effects of x-rays upon the hypothalamus.

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HYPOTHYROIDISM AS AN INBORN ERROR OF METABOLISM

JAMES H. HUTCHISON, M.D., F.R.C.P. AND
E. M. McGIRR, M.B., M.R.C.P.

The University Department of Child Health, Royal Hospital for Sick Children, Glasgow, and the University Department of Medicine, Royal Infirmary, Glasgow, Scotland

CRETINISM in the United Kingdom is commonly sporadic and athyroidic; iodine intake is adequate but functional thyroid tissue is absent. In areas of endemic goiter, cretins are born with goiters; functional thyroid tissue is available initially but there is an insufficient supply of iodine to allow the thyroid gland to keep the fetus or infant in hormonal balance; the thyroid epithelium undergoes exhaustion atrophy after prolonged over-stimulation by thyrotropin. The pathogenesis of these two classic types of hypothyroidism is fairly clear. Within recent years, a third type of hypothyroidism has been encountered consequent upon the therapeutic use or the accidental ingestion of substances with a goitrogenic action such as para-amino-salicylic acid (1), thiocyanate (2), sulphonamides and the thiouracils (3, 4), the 2-mercaptoimidazoles (5) and resorcinol (6, 7).

From time to time hypothyroid children are seen who cannot be fitted into one of these well defined types. The literature contains several reports that *goitrous cretinism*, which appears usually to be *familial*, may occur outside areas of endemic goiter (8–13). Furthermore, the pathogenesis in these various cases seems not to have been identical. *Juvenile myxedema*, which develops after the first year of life, which may be goitrous or non-goitrous, and which is not obviously familial, has long been recognized but its etiology remains unexplained; Braid (14) has regarded it as postinfective but the evidence for this is unconvincing. It seems likely that these unexplained cases are worthy of study by modern radioisotope techniques which might be expected to throw some light on the problem of their pathogenesis and which might, indeed, increase our understanding of thyroid physiology.

We have already published the results of radioactive-iodine (I^{131}) studies in 12 cases of goitrous hypothyroidism in children (13). In the present paper, we describe the clinical findings and discuss the significance of the

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radioiodine results in these patients and in an additional patient who also showed myxedematous characteristics in association with thyroid enlargement.

CLINICAL MATERIAL

For descriptive purposes the patients may be divided into three groups:

Group 1 comprises Cases 1–7 and Case 13 . . . 3 males and 5 females, all members of one family group (Table 1).

Group 2 comprises Cases 8–10 . . . 2 males and 1 female, unrelated to each other or to the patients in Groups 1 and 3.

Group 3 comprises Cases 11 and 12 . . . both female children of a common mother but having different fathers.

For the convenience of those who may wish to refer to our earlier paper, the patients described here have been given the same serial numbers. The additional patient has been referred to as Case 13, although she is a member of the same family group as Cases 1–7.

Group 1

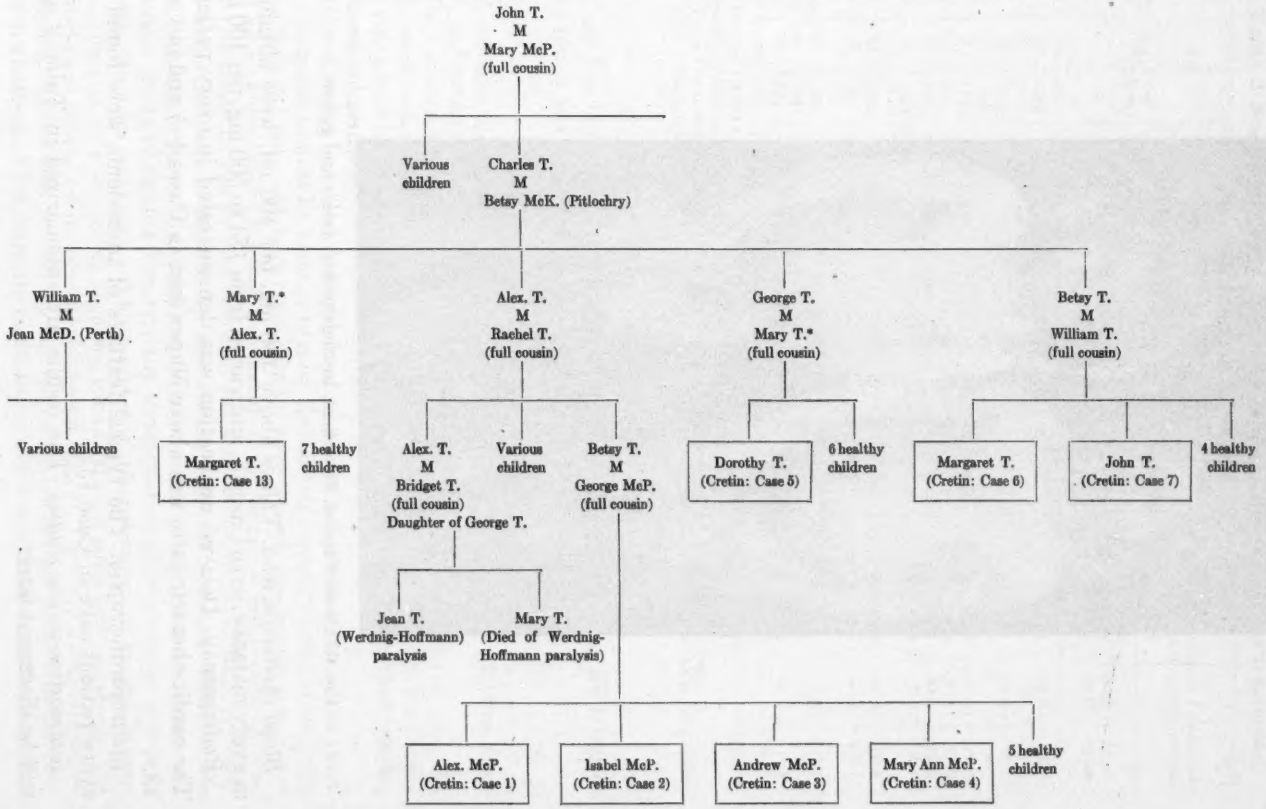
The patients in this group were of intinerant tinker stock living a nomadic existence in Argyllshire and the Isle of Islay, Scotland, and originating in Ireland. A family tree, summarizing such information as was available, is shown in Table 1. The amount of consanguinity is astonishing and probably only to be found among tinker-folk.

Age at onset. The ages of the patients at the time of onset of myxedema or goiter could not be determined with accuracy. It would appear that development took place normally during the first three years of life in Cases 1–4 and Case 13, whereas in Case 5 abnormal development, but probably not the goiter, was noted before the age of 1 year. The early history in Cases 6 and 7 was not obtainable.

Clinical features. In all of these patients, except in Case 13, the myxedematous changes were notable at a glance (Fig. 1). In addition to a striking family likeness, there was the typical coarse, rather ugly facies of myxedema, as well as supraclavicular pads, scanty eyebrows and dry lusterless hair. All of the patients were dwarfed and mentally defective. Nevertheless, in none of the patients were the signs of hypothyroidism as gross as they would have been in untreated sporadic or endemic cretins of comparable ages. In Case 13, the evidence of hypothyroidism was not obtrusive, although easily obtained by careful clinical observation. In each of the 8 patients there was a very obvious but not gross goiter. In Case 3 there was, in addition to myxedema, a mild spastic paraplegia, for which tendon-lengthening had been performed.

TABLE 1. FAMILY TREE OF GROUP 1 CASES

(The original Mr. T. came from Ireland some 160 years ago and settled as a blacksmith at Southend, Kintyre, Scotland)



* Mary (mother of Margaret, Case 13) was an aunt of Mary (mother of Dorothy, Case 5).

FIG. 1. Cases 2 and 4. Dwarfism, myxedematous facies and goiters.

Blood cholesterol level. Values above 180 mg. per 100 ml. were obtained in every instance, save Case 13, and varied from 184 to 500 mg. per 100 ml.

Radiography. Delayed ossification was demonstrated in every patient. The cardio-thoracic ratio was above 50 per cent in Cases 1–5 and in Case 13.

Electrocardiography. The typical features of myxedema were found in every patient save in Case 13.

Radioactive-iodine studies. The results are summarized in Table 2 and will be discussed later.

TABLE 2. SUMMARY OF I^{131} STUDIES IN 13 CASES OF GOITROUS HYPOTHYROIDISM

Case No.	Sex	Age (yrs.)	Bone age (yrs.)	Percentage of I^{131} in thyroid at time of max. measured uptake and at 24 hrs.*			Percentage of I^{131} per liter of plasma at 48 hrs.			Percentage of I^{131} excreted in urine in various periods		
				Max. uptake	Time to max. uptake	Uptake at 24 hrs.	T.P.I.†	P.B.I.†	B.E.I.†	0–24 (hrs.)	6–24 (hrs.)	24–48 (hrs.)
1	M	$15\frac{7}{12}$	7–8	79	3 hrs.	24	0.95	0.50	—	59.6	50.3	27.0
2	F	$11\frac{7}{12}$	4–5	88	2 hrs.	—	—	—	—	—	—	—
3	M	$10\frac{7}{12}$	4–5	80	6 hrs.	77	—	—	—	27.0	14.0	14.0
4	F	$8\frac{6}{12}$	3–5	90	1 hr.	38	—	—	—	—	—	—
5	F	$7\frac{7}{12}$	2–3	—	—	49	1.02	0.49	—	46.0	40.5	19.8
6	F	20	16–17	—	—	44	0.45	0.10	—	35.6	27.1	23.0
7	M	16	13	40	2 hrs.	36	1.78	1.12	—	50.3	38.4	17.6
8	M	$4\frac{3}{12}$	under 1	—	—	75	1.54	1.00	—	20.0	13.0	5.4
9	M	$2\frac{9}{12}$	1	—	—	23	1.68	0.58	—	55.0	52.8	14.4
10	F	4	under 1	—	—	12.5	1.28	0.87	—	48.0	27.6	4.0
11‡	F	$6\frac{1}{2}$	3	75	5 hrs.	38	1.35	0.49	0.36	—	—	—
12‡	F	$3\frac{1}{2}$	3	74	5 hrs.	31	1.32	0.40	0.25	—	—	—
13	F	$12\frac{1}{2}$	10	23	5 hrs.	21	0.32	0.08	0.09	24.5	21.2	9.8

* In Cases 1–4 and 8, potassium thiocyanate (1–2 Gm.) was given orally at 48 hrs., 4 hrs., 24 hrs., 4 hrs., and 48 hrs., respectively, without measurable effect on the thyroid content of I^{131}.

† T.P.I. = Total Plasma Iodine. P.B.I. = Protein-Bound Iodine. B.E.I. = Butanol-Extractable Iodine.

‡ After 6 mos. and 8 mos. of thyroid medication, respectively, treatment was stopped 2 mos. before I^{131} studies.

Illustrative Cases

Case 1 is typical of the group. Case 13 is atypical, in that all features were much less striking.

Case 1

A boy aged $15\frac{7}{12}$ years was admitted to the Royal Hospital for Sick Children, Glasgow, on September 26, 1951.

He was born spontaneously after a normal pregnancy, the fifth child of nine. His birth weight was 8 pounds and he seemed healthy. He is said to have walked at the age of 1 year and to have begun to say words at the age of 13 months. He had had measles, pertussis and mumps.

He had always been "slow" at school and progressed only three classes in ten years. He was unable to write his name or to read. He had only recently started to play with other boys and only with boys much younger than himself. His parents were aware that his neck was swollen but they were unable to say when this first became obvious.

On admission he was dwarfed (136 cm.) and underdeveloped (35.72 Kg.), obviously dull-witted and slow to react to his surroundings or to show emotion. His skin was pale, with a faint yellow tinge, and was thick and coarse. His hair was dry, coarse and lusterless. His facial features were typically myxedematous. Supraclavicular pads were present. His voice was husky and his tongue was enlarged and much fissured. His external genitalia were not grossly abnormal but axillary and pubic hair was absent. The thyroid gland showed diffuse smooth enlargement (Fig. 2). There was a moderate degree of anemia: Hb. 9.8 Gm. per 100 ml. and red cells 3,130,000 per cu.mm. *Radiography* showed a bone-age of 7–8 years; the cardio-thoracic ratio was 52 per cent. *Electrocardiog-*

FIG. 2. Case 1. Myxedematous facies and goiter.

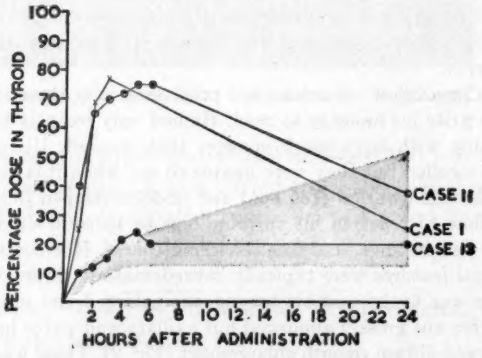


FIG. 3. Uptake of radioiodine by thyroid gland in Cases 1, 11 and 13. Stippled area depicts normal pattern of I^{131} uptake in the West of Scotland.

raphy showed low voltage in all leads, with a rate of 65 per min. *Blood cholesterol* concentration was 200 mg. per 100 ml.

I^{131} studies were carried out by the techniques previously described (13). The dose of I^{131} was 31 μc. The results are shown in Table 2 and Figure 3. The. The maximum observed uptake of radioiodine by the thyroid gland was 79 per cent of the ingested dose after three hours. At twenty-four hours the I^{131} content of the thyroid gland had fallen to 24 per cent, and at forty-eight hours to 14 per cent. This level was uninfluenced by the oral administration of 2 Gm. of potassium thiocyanate. The total plasma radioactivity after forty-eight hours was 0.95 per cent of the ingested dose per liter of plasma, of which 0.50 per cent per liter was in the protein-bound fraction. The urinary excretion figures were 59.6 per cent of the radioiodine dose in the 0–24 hour period, 50.3 per cent in the 6–24 hour period, and 27.0 per cent in the 24–48 hour period.

Treatment and progress. After four months of treatment with desiccated thyroid, 1½ gr. daily, he was vastly improved mentally and physically. He had grown 3 cm., the blood cholesterol level was 65 mg. per 100 ml., ossification was considerably advanced and the electrocardiogram was normal.

Case 13

A girl aged 12½ years was admitted to hospital on December 24, 1952.

She was born spontaneously after a normal pregnancy, the fourth child of eight. She seemed to be healthy. She had walked and talked at about the age of 18 months. She had had measles, pertussis and mumps.

Her parents had regarded her as "normal," although small in stature, but they did admit that her school performance was "not good." They had not noticed any swelling of the neck.

On admission she was dwarfed (128 cm.) and under-developed (26.56 Kg.). She was obviously slow mentally and completely illiterate. Her skin was pale, thick and coarse, and her hair was dry and lusterless. The facial features were mildly myxedematous. Supraclavicular pads were just visible. Her voice was rather husky. The tongue appeared normal. Secondary sexual development had not begun. There was a soft diffuse goiter. A mild anemia was present: Hb. 11.9 Gm. per 100 ml.; red cells 4,300,000 per cu.mm. *Radiography* showed a bone-age of 10 years; the cardio-thoracic ratio was 64 per cent. *Electrocardiography* showed an inverted T_3 but none of the features of hypothyroidism. *Blood cholesterol* concentration was 162 mg. per 100 ml.

I^{131} studies. The dose of I^{131} was 50 μc. The results are shown in Table 2 and Figure 3. The maximum observed uptake of radioiodine was 23 per cent after five hours. At twenty-four hours the level was 21 per cent. The total plasma radioactivity after forty-eight hours was 0.32 per cent of the dose per liter, of which 0.08 per cent was in the protein-bound fraction. The butanol-extractable iodine was 0.09 per cent per liter. The urinary excretion figures were 24.5 per cent of the dose in the 0–24 hour period, 21.2 per cent in the 6–24 hour period, and 9.8 per cent in the 24–48 hour period.

Treatment and progress. There was a striking mental and physical response to treatment with desiccated thyroid, 1½ gr. daily.

Group 2

The patients in this group (Cases 8–10) were 3 unrelated children, all resident in Glasgow.

Age at onset. All 3 patients were referred to the hospital out-patient

clinics because of delay in walking or talking. The retardation was first appreciated by the parents at about the age of 2 years. Thyroid enlargement had not been noted in any patient.

Clinical features. The facies of these patients were only mildly myxedematous in appearance, although the supraclavicular pads were rather striking. The hair and eyebrows were normal. All the patients were dwarfed and retarded mentally. The thyroid gland in each patient was palpable and visible; it was obviously enlarged in relation to its functional inefficiency.

Blood cholesterol level. The values were 150, 260 and 236 mg. per 100 ml. in Cases 8, 9 and 10, respectively.

Radiography. Ossification was delayed in each patient. The cardiothoracic ratio was 57 per cent in Case 10, and 50 per cent in Cases 8 and 9.

Electrocardiography. Low voltage tracings were obtained from each patient.

Radioactive-iodine studies. The results are summarized in Table 2.

Illustrative Case

Case 8

A boy aged $4\frac{3}{12}$ years was admitted to the hospital on February 28, 1952.

He was born spontaneously after a normal pregnancy, the second child of two. His birth weight was 8 pounds and he seemed healthy. He sat up at the age of 9 months, walked at 2 years and began to say words at $2\frac{1}{2}$ years.

At the age of 3 years he was noted to be "slow," so that even at the time of admission he had only a few simple words and he walked badly. His appetite had always been small and he had always been constipated. He had no control over his bladder or bowel.

On admission he was dwarfed (68 cm.), under-developed (10.56 Kg.) and obviously considerably retarded mentally. He adopted a lordotic posture with the hips and knees slightly flexed (Fig. 4). He was pot-bellied, and his skin was pale, thick and coarse. His hair and eyebrows were normal, and his facial features were not grossly cretinous. The tongue was not obviously abnormal. The thyroid gland was readily visible and palpable. There was a mild anemia: Hb. 11.9 Gm. per 100 ml.; red cells 3,600,000 per cu.mm. *Radiography* showed a bone-age of under 1 year; there was no cardiac enlargement. *Electrocardiography* showed low voltage. *Blood cholesterol* concentration was 150 mg. per 100 ml.

I^{131} studies. The dose of I^{131} was 30 μc. The results are shown in Table 2. The uptake of radioiodine by the thyroid gland was measured only after twenty-four hours (75 per cent of the dose) and after forty-eight hours (70 per cent). The total plasma radioactivity after forty-eight hours was 1.54 per cent of the dose per liter, of which 1.0 per cent was in the protein-bound fraction. The urinary excretion figures were 20.0 per cent of the dose in the 0–24 hour period, 13.0 per cent in the 6–24 hour period, and 5.4 per cent in the 24–48 hour period.

Treatment and progress. After treatment with desiccated thyroid, 2 gr. daily for one year, he was vastly improved both mentally and physically. He was proving educable at school and his height had increased by 23 cm. A radiograph of the wrist showed two carpal centers and the radial epiphysis, where previously there had been none.

FIG. 4. Case 8. Dwarfed, hypothyroid patient alongside normal child of same age. (By courtesy of the publisher—from Hutchison, J. H. *in* "Paediatrics for the Practitioner," edited by W. Gaisford and R. Lightwood. In press, London, Butterworth & Co.)

Group 3

The first patient in this group (Case 11) was the mother's third child by her first husband; the second patient (Case 12) was her first child by her second husband. Her third child by her second husband was also a cretin but we were not able to examine him. The mother was of tinker stock from Perthshire and Angus. The clinical descriptions which follow are from the case notes supplied by Professor J. L. Henderson of Dundee, who referred the patients to us after they had been under thyroid treatment for six months and eight months, respectively.

Age at onset. Failure of normal development was noted before the age of 2 years. In the case of the third cretin in this family, a male child, the diagnosis was made by Professor Henderson when the infant was 3 months old; the parents refused to allow radioisotope studies in this patient.

Clinical features. In contrast with the patients of Groups 1 and 2, these children when first seen in Dundee were typical untreated cretins with ugly coarse flat facies, large tongues, supraclavicular pads, pot-bellies (umbilical hernia in Case 12), severe dwarfism and mental deficiency (Fig. 5). In each patient there was an obvious although not very large goiter.

Blood cholesterol level. Hypercholesterolemia was present in both patients prior to treatment.

Radiography. Ossification was markedly delayed. Teleradiographs were not made prior to treatment.

Electrocardiography. The tracings were normal.

Radioactive iodine studies. (Table 2.) These were carried out in Glasgow September 16–18, 1952, after thyroid treatment had been stopped for two months.

Illustrative Case

Case 11.

A girl aged 6 years was admitted to Dundee Royal Infirmary under the care of Professor Henderson on January 21, 1952.

She was born spontaneously after a normal pregnancy, the third and last child by the mother's first husband. She had always been small and backward. She began to walk and talk at the age of 2 years but had made little progress since then. She had always had a large belly and had never had control over her bladder and bowel.

On admission she was dwarfed (90.2 cm.), under-developed (14.15 Kg.) and mentally defective. She stood with kyphosis and lordosis and her abdomen was protuberant. Her skin was pale, dry and coarse. She had typical cretinous features (Fig. 5) and a large tongue. Supraclavicular pads were marked. There was a visible diffuse smooth goiter. Anemia was moderate: Hb. 8.7 Gm. per 100 ml.; red cells 2,800,000 per cu.mm. *Radiography* showed a bone-age of 2 years. *Electrocardiography* revealed normal tracings. *Blood cholesterol* concentration was 210 mg. per 100 ml.

I^{131} studies. The dose of I^{131} was 126 μg. The results are shown in Table 2 and Figure 3. The peak-uptake by the thyroid gland was 75 per cent of the dose after five hours. At twenty-four hours the I^{131} content of the thyroid gland had fallen to 38 per cent. The total plasma radioactivity after forty-eight hours was 1.35 per cent of the dose per liter of plasma, of which 0.49 per cent per liter was in the protein-bound fraction. The butanol-extractable radioiodine was 0.36 per cent of the dose per liter of plasma. Urinary excretion studies were omitted because of difficulty in urine collection.

Treatment and progress. We have been informed by Professor Henderson that this patient responded very satisfactorily to thyroid treatment and is no longer recognizable as a cretin.

Discussion

Clinical observations

The 3 patients of Group 2 fall typically into the textbook classification of juvenile myxedema, but distinction between cretinism and juvenile myxedema is obviously artificial in patients of Groups 1 and 3. To avoid such artificial distinctions, which fail to take into account the natural

FIG. 5. Cases 11 and 12. Half-sisters, showing typical features of untreated cretinism

development of the condition and which are, to some extent, based on the clinical history and thus on the variable powers of observation of the parents and medical attendants, we consider the inclusive term "hypothyroidism" to be preferable.

The degree of hypothyroidism in our 13 patients varied considerably. In Cases 11 and 12, the clinical picture was that of untreated sporadic cretinism in every respect save for the presence of goiter. In Cases 1–7 (of one family group) and 8–10 (unrelated) hypothyroidism was moderate in degree. In Case 13 (of the same family group as Cases 1–7) it was mild. The size of the thyroid glands also varied widely. From such clinical evidence alone, it is reasonable to infer that the thyroid glands of these children had, at one time at any rate, produced a variable amount of thyroid hormone or of a precursor which had a similar action. Hubble (12) has suggested that the clinical records of goitrous cretinism, both endemic and nonendemic, often denote a relative and progressive, rather than an abso-

lute, insufficiency of thyroid hormone. The marked response to treatment with thyroid which occurred in every one of our cases precludes the possibility of normal hormone production in the presence of tissue insensitivity.

Radioiodine studies

Our observations with radioactive iodine have made it possible to give a tentative explanation of how these children, who were not suffering from deprivation of iodine or from the action of any known goitrogen and who certainly did not lack thyroid glands, came to be myxedematous.

The normal pattern of the uptake of radioiodine by the thyroid gland in the West of Scotland as measured by our technique is shown in Figure 3. The peak-uptake does not usually occur until at least twenty-four hours after the oral ingestion of radioiodine, and the 24-hour value does not exceed 52 per cent of the dose. In Cases 1–4, 11 and 12, in which serial measurements were made, there was an abnormally rapid and high uptake (Table 2, Fig. 3). In only 2 patients (Cases 3 and 8), however, was the radioiodine content of the thyroid glands still above the normal range after twenty-four hours. It is obvious that by then the glands were already releasing their radioiodine back into the circulation. That it was in organic combination was proved in Cases 1–4 and 8 by the fact that the amounts of radioiodine in the thyroid glands were not influenced by the oral administration of potassium thiocyanate after different intervals. Potassium thiocyanate is known to cause the abrupt discharge of inorganic iodide from the thyroid gland (15).

Measurement of plasma radioactivity forty-eight hours after the ingestion of radioiodine confirms these findings. In 8 of 10 patients the total radioactivity was above 0.7 per cent of the dose per liter of plasma, which is the maximum normal level reported in the United Kingdom (16). Even more significant are the protein-bound I^{131} levels. In hypothyroidism, protein-bound activity is usually negligible, and in only 2 of their euthyroid subjects did Goodwin *et al.* (16) find levels above 0.2 per cent of the dose per liter. In 7 of 10 patients we found levels in excess of 0.4 per cent per liter. On the assumption, which is usually true, that the protein-bound iodine obtained by trichloracetic-acid precipitation is a measure of the thyroxine content of the plasma, a level of protein-bound I^{131} above 0.4 per cent per liter has been taken to indicate a state of hyperthyroidism (17). However, Man *et al.* (18) have shown that some diiodotyrosine may be precipitated by this method. We, therefore, considered the possibility that diiodotyrosine, not thyroxine, was the basis of the protein-bound activity in our patients. In Cases 11–13, we examined the plasma by a butanol-extraction method (19) which removes inorganic iodide and diiodotyrosine, but not thyroxine-like iodine, from the butyl alcohol

extract. In each case radioactivity persisted in the extract, although in Cases 11 and 12 it was less than that precipitated by trichloracetic acid. The chemical basis of this residual radioactivity is unknown, but the presence of substantial amounts of protein-bound radioiodine in patients who were hypothyroid and yet responded well to desiccated thyroid given orally, suggests that it might not be the thyroid hormone.

In contrast with the rapid uptake of I^{131} by the gland and the findings in plasma, the total amounts of radioiodine excreted in the urine in twenty-four and forty-eight hours in the majority of cases examined were such as would be expected in ordinary adult myxedema due to thyroid atrophy. The 24-hour excretion levels were in the widely variable normal and hypothyroid range found in the United Kingdom (16), but in 6 of 9 patients the 24–48 hour figures were at the high levels characteristic of adult myxedema (20, 21).

The high urinary excretion values in the presence of high thyroidal uptake contrast with the low excretions found in thyrotoxic subjects, even in those in whom there is a considerable decrease of thyroidal activity by twenty-four hours. This observation suggests that the organic iodine compound released into the circulation is different in the two conditions, and in our patients was not being used but was being excreted. Oliver and Ellis (22, 23) have analyzed the urinary excretion curves of Cases 1–12. They remark that they have found similar excretion curves, with the exception of that of Case 10, in patients who are receiving, or who have recently received thiouracil; and they consider that the type of curve suggests an incomplete thyroid action similar to that obtained with thiouracil.

The opinion that our patients were not producing the thyroid hormone receives support from the chromatographic studies which Dr. J. Gross and Mrs. R. Pitt-Rivers (24) of the National Institute of Medical Research, London, performed on samples of plasma from Case 5 (dose of I^{131}, 200 μc.). They confirmed that butanol extracts of the plasma contained radioactivity. Samples were examined on a kieselguhr column and, though the basis of the radioactivity was not determined, the absence of thyroxine and triiodothyronine was confirmed.

The unexpected formation of protein-bound I^{131} in myxedema was first reported by McConahey *et al.* (25), who tentatively suggested extrathyroidal organic binding of iodine. Riggs (26), discussing their finding, considered a more likely alternative was the possibility that scanty but very active foci of functional thyroid tissue, under intense stimulation with thyrotropin, and with no storage capacity for organically bound iodine, should secrete into the blood-stream "very limited" amounts of protein-bound I^{131}. Blom and Terpstra (27), commenting on the presence of important amounts of protein-bound I^{131} (0.43 per cent and 0.19 per cent

of the dose per liter of plasma at forty-eight hours) in 2 patients with clear-cut hypothyroidism following subtotal thyroidectomy for thyrotoxicosis, favored this hypothesis, the essential feature of which is the lack of a diluting pool of iodine in the gland. They presented theoretical evidence in its support, but disagreed with the suggestion of the "very limited" appearance of organic radioactive iodine in the blood stream. There is no reason to doubt that the thyroid remnants in their patients were potentially able to produce the thyroid hormone and, theoretically, in amounts sufficient to explain the substantial protein-bound I^{131} values. The presence of thyroxine was not conclusively proved, however, and we are left to wonder whether their protein-bound I^{131} may not have consisted of more than one compound.

It appears to us desirable that, when anomalous or unexpected results are found in protein-bound I^{131} (12, 13, 16, 28, 29) or other I^{131} investigations, the patients should be studied as fully and in as much detail as possible, and every effort should be made to determine the true identity of the substances being measured. Robbins *et al.* (30) have recently found in the serum of 2 patients with thyroid carcinoma who received therapeutic doses of radioiodine, an iodine component entirely precipitable with the serum proteins and only partially soluble in butyl alcohol, which is neither thyroxine nor triiodothyronine.

Reconsideration of our findings (the high uptake of I^{131} by the enlarged thyroid glands, the urinary excretion results, the high protein-bound I^{131} values, and especially the proven absence of thyroxine or triiodothyronine in one of the cases), even in the light of the hypothesis of Riggs (26) and Blom and Terpstra (27), leaves us convinced that the organic iodine compound released into the circulation in our cases was not the thyroid hormone. In consequence of the qualitative defect of hormone synthesis which occurred, we visualize secondary increased anterior-pituitary activity and excess production of thyrotropin leading to thyroid gland enlargement, increased avidity for I^{131}, and the very rapid release into the circulation of an unidentified organic iodine compound which was readily excretable.

Comparison with other cases

Excessive avidity for iodine has been a feature common to all the non-endemic goitrous cretins in whom radioactive iodine studies have been reported (8–13). More detailed comparison, however, reveals significant differences between these patients. In Stanbury and Hedge's patients (10) the the radioiodine so rapidly accumulated by the thyroid glands was even more rapidly discharged by potassium thiocyanate, a fact taken to indicate that in their patients the thyroid gland was unable to combine inorganic

iodide into a protein-complex. Hamilton. *et al.* (8) found in 2 hypothyroid children with goiters that a thyroxine-like iodine compound was present in the thyroid glands after operative removal. Though it seemed that the glands were able to make thyroxine, it was thought (but without the evidence of blood studies) that they were unable to release it into the circulation. In 1 of the 4 familial goitrous cretins described by Hubble (12) it was conclusively shown that not only did the thyroid gland contain thyroxine after operative removal but also that it could liberate thyroxine into the plasma. It must be assumed that in this patient either the amount of thyroxine was inadequate or the deiodination of thyroxine to give triiodothyronine was defective. In our patients it has not been possible to study the chemical basis of the radioactivity in the thyroid glands because operation was not considered to be justifiable, but the results of the radioiodine studies indicate that the protein-bound iodine compound which the glands were releasing into the circulation in comparatively large amounts was *not* thyroxine or triiodothyronine.

It seems clear that the lack of thyroxine or triiodothyronine in these several groups of patients arose from a defect which occurred at different stages in the synthesis of the thyroid hormone. This has been expressed as a four-stage reaction: (i) the conversion of iodide to iodine, which is generally regarded as an enzymatic oxidation; (ii) the iodination of tyrosine to diiodotyrosine, (iii) the oxidative coupling of two molecules of diiodotyrosine to give one molecule of thyroxine, which may or may not be due to enzymatic action; and (iv) the deiodination of thyroxine to give triiodothyronine, probably also the result of enzyme action (24). The nature of the enzymes responsible for this series of reactions is unknown. It is known, however, that various goitrogenic drugs may inhibit hormone synthesis at different stages. Thus, thiocyanate appears to interfere with the iodine-concentrating mechanism of the thyroid gland (15, 31, 32), whereas the thiouracils and the sulphonamides seem to interfere with the conversion of inorganic iodide to diiodotyrosine (4, 33), although they differ in the primary biochemical reactions responsible for their effects (31, 34). It seems probable that enzymatic or other biochemical defects may, at times, occur spontaneously at various levels in hormone synthesis, just as they can readily be produced artificially by drugs.

Genetic considerations

Most goitrous cretins reported from nonendemic areas have had similarly affected siblings. Lerman *et al.* (9) described detailed investigation of 2 brothers. Stanbury and Hedge (10) reported 4 cretinous siblings with goiters, whose parents were first cousins. Hubble (12) described a family of 4 cretins in one generation. We have described here 8 hypothyroid children

from one family group (Table 1) and 2 cretinous half-sisters among whose 4 other siblings was another known cretin. These reports indicate clearly that the etiology of hypothyroidism in childhood may, on occasion, be genetically determined. The family tree described in Table 1, with its remarkable history of consanguineous matings, makes it very probable that the hypothyroidism in this family was due to a recessive autosomal gene, and was a biochemical anomaly of the type originally studied by Garrod (35). He recognized the frequency of consanguineous marriages in conditions such as alkaptonuria and congenital porphyrinuria long before a high rate of consanguinity was proved to be the best proof of recessive inheritance in man. Garrod's prophecy in regard to inborn errors of metabolism that "those hitherto recognized are doubtless but samples of a far larger group of anomalies so far undetected" has already been fulfilled, for example, in the familial aminoacidurias such as Wilson's hepatolenticular cirrhosis, the Lignac-Fanconi syndrome and cystinosis. It would seem that some forms of cretinism and myxedema in childhood must be added to the growing list of inborn errors of metabolism. To quote Garrod again, "they are of great interest—for they are, so to speak, Nature's experiments, by the study of which valuable light is thrown upon the normal working of the metabolic processes in man."

SUMMARY

The clinical features of 13 hypothyroid children with enlarged thyroid glands are described, and the results of radioactive iodine (I^{131}) studies discussed.

None of the patients came from an area of endemic goiter. Eight were members of one family group; 2 were members of a second family; and 3 were unrelated to either family or to each other. Their ages ranged from 2 years and 9 months, to 20 years; 5 were males and 8 females.

The age of the patient at the time of onset of recognizable features varied from the first to the third years of life; the severity of hypothyroidism varied from the picture of untreated cretinism to one of mild myxedema; and the size of the goiters varied considerably.

Reported cases of goitrous cretinism show a high familial incidence. The family tree of our larger family group suggests that the hypothyroidism in these cases is sometimes an inborn error of metabolism due to a recessive autosomal gene.

The results of the radioactive iodine studies show that nonendemic goitrous cretinism or juvenile myxedema may occur in the presence of a thyroid gland which is able and eager to take up iodine, to link it to protein, and to release it into the circulation in an organic form which is not thyroxine nor triiodothyronine.

It is postulated that in such cases there is an intrinsic defect in the synthesis of the thyroid hormone. Study of similar reported cases indicates that the deficiency may be quantitative or qualitative, and that the apparently spontaneously occurring qualitative defects in hormone synthesis may occur at different levels.

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MEASUREMENT OF PLASMA CHORIONIC GONADOTROPIN LEVELS IN MEN RECEIVING THIS HORMONE: AN APPLICATION OF THE AUGMENTING EFFECT OF PLASMA*

ROBERT B. LEACH, M.D., ICHIRO TOKUYAMA, M.D.† AND WILLIAM O. MADDOCK, M.D., PH.D.

The Department of Medicine, Wayne University College of Medicine, and the Endocrine Clinic of the City of Detroit Receiving Hospital, Detroit, Michigan

ALTHOUGH chorionic gonadotropin has been measured in the blood of pregnant women, it has not been measured in the blood of nonpregnant patients receiving the hormone. Studies of the fate of administered gonadotropin have been limited to the determination of urinary excretion rates (1, 2). Apparently it has been presumed that the amounts present in the blood following therapeutic doses would be too low for measurement with present methods of assay. Therefore, when we observed that chorionic gonadotropin administered intramuscularly could be detected in the plasma by a standard bioassay method, studies of plasma chorionic gonadotropin levels were undertaken in 10 men receiving the hormone.

METHODS AND OUTLINE OF STUDY

Assays of plasma chorionic gonadotropin levels were performed in immature Sprague-Dawley female rats, using increase in uterine weight as the criterion of hormonal activity. The reasons for using uterine, rather than ovarian, weight are discussed in another report (3). The total amount of plasma to be administered to each rat was diluted to 6 cc. with tap water and 1 cc. was administered twice daily for three days. Twenty-four hours after the last injection the rats were killed and the uteri were weighed after expression of any intraluminal fluid. The minimal amount of plasma necessary to produce a response was approximated by preliminary assays.

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† Fellow in Medicine, on leave as Instructor in Medicine, University of Kyushu, Fukuoka City, Japan.

Measurements were then repeated using amounts of plasma necessary to give responses falling within, or bracketing, the sensitive range of the assay. For the majority of plasma samples, two or more dilutions of plasma were assayed, using at least 3 rats per dilution.

Augmentation of chorionic gonadotropin activity by plasma: Initial studies revealed surprisingly high levels in the patients' plasma, so high as to suggest that some substance in plasma was capable of increasing the activity of the hormone as measured in the assay animals. Experiments which are reported in detail elsewhere (3) demonstrated that mixing unaltered human plasma, obtained from untreated individuals, with an aqueous solution of chorionic gonadotropin, produced a tenfold increase in biologic activity as measured in the assay rats. When mixed with plasma, 0.1, 0.2 and 0.5 unit of hormone produced responses similar to those produced by 1.0, 2.0 and 5.0 units of hormone in water (Table 1). Since augmentation did not occur when plasma and hormone were administered at separate sites in the assay rats, and since augmentation was produced by other substances such as eggwhite and polyvinylpyrollidone, it was concluded that prolongation of absorption from the site of injection was the probable mechanism of augmentation of chorionic gonadotropin activity (3).

TABLE 1. THE AUGMENTING EFFECT OF THE *in vitro* ADDITION OF HUMAN PLASMA ON THE ASSAY OF CHORIONIC GONADOTROPIN IN RATS

Material	International units per rat	Assay rats	
		Uterine weight (mg.)	No. of rats
Chorionic gonadotropin in aqueous solution ("A.P.L.")*	0.5	36	12
	1.0	56	15
	2.0	107	16
	5.0	137	7
	10.0	110	4
	50.0	114	4
Chorionic gonadotropin mixed with 0.1 to 3.0 cc. of plasma obtained from untreated individuals†	0.1	48	38
	0.2	92	29
	0.5	137	18
Uninjected controls		30	27

* Entirely similar results were obtained with the International Standard for Chorionic Gonadotropin obtained from the U.S.P. Reference Standards (3).

† These values are the averages of assays performed using plasma obtained from 5 untreated subjects. The individual figures for the plasma samples of each patient are presented in Table 2, reference (3).

Calculation of plasma levels: Plasma levels of chorionic gonadotropin were calculated by comparing effects produced by the patients' plasma samples with effects produced by known amounts of chorionic gonadotropin. To allow for the augmenting action of plasma, values for chorionic gonadotropin mixed with plasma from untreated individuals (Table 1), rather than the values for the aqueous solutions of chorionic gonadotropin, were used for the comparison. Since 0.2 unit of hormone in plasma gives a definite uterine weight response, and since as much as 6 cc. of plasma can be administered to each rat, as little as 0.03 unit of chorionic gonadotropin per cc. of plasma can be detected. Both small and large amounts of plasma produce similar degrees of augmentation (3); therefore, no correction has been made for the fact that different concentrations of plasma were present in the different dilutions tested.

Specificity of the assay: The assay end-point used in this study, uterine weight of intact immature rats, is not specific for chorionic gonadotropin. However, the following considerations indicate that chorionic gonadotropin was the hormone measured in these studies: 1) No hormonal effect has been observed from the administration to rats of as much as 6 cc. of plasma obtained from untreated normal individuals or untreated patients having either hypogonadotropic or hypergonadotropic hypogonadism. 2) The administration to castrated rats of plasma obtained from a patient receiving chorionic gonadotropin produced no effect—even though amounts of plasma eight times that necessary to produce a minimal response in intact rats were employed. 3) The administration to intact rats of increasing amounts of plasma obtained from patients during therapy, produced dose-response curves typical of chorionic gonadotropin. With amounts of plasma five to ten times the minimal amount necessary to produce a uterine response, enlargement, luteinization and hyperemia of the ovaries occurred. 4) The patients' endogenous pituitary gonadotropins did not appear to affect the assay, since similar results were observed in individuals having hypogonadotropic and hypergonadotropic hypogonadism.

Patients: Of the 10 men receiving chorionic gonadotropin, 2 had panhypopituitarism, 5 had hypogonadotropic eunuchoidism, 2 had primary testicular failure and 1 had normal testicular function. Urinary gonadotropin excretion, assayed by the method of Jungck *et al.* (4), was low in the first 7 patients, high in the 2 patients having primary testicular failure and normal in the patient having normal testicular function.

Therapy: Each of the patients received 5,000 units of chorionic gonadotropin[1] intramuscularly three times weekly. A sufficient amount of aqueous

[1] "A.P.L.," generously supplied by Ayerst, McKenna and Harrison, Ltd., was used throughout these studies.

diluent was added to the dry powder hormone preparation so that 2 cc. of solution contained 5,000 units of chorionic gonadotropin. In some of the patients, other dosage schedules were also employed (Table 4).

Intravenous administration of chorionic gonadotropin: Following intramuscular administration, relatively high plasma levels of chorionic gonadotropin were maintained for several days. To determine whether this was due to prolonged absorption from the site of intramuscular injection, or to slow disposal after absorption, plasma levels were studied in a patient following intravenous administration of the hormone. For intravenous administration, the hormone was diluted to 10 cc. with isotonic saline and slowly injected over a five-minute period.

Intramuscular administration of chorionic gonadotropin in 30 per cent polyvinylpyrollidone (PVP): To determine if PVP, which enhances the activity of chorionic gonadotropin in rats (3), could also augment the hormone in human subjects, chorionic gonadotropin in a 30 per cent solution of PVP, 2,500 units per cc., was administered intramuscularly to 2 hypogonadotropic eunuchoids. The activity of the hormone in PVP was compared with that of the usual aqueous solution of hormone, by determining the ability of the respective preparations to stimulate Leydig-cell function. The following two criteria were used to evaluate Leydig-cell function: 1) urinary estrogen excretion, and 2) the subjective response of the patient. Urinary estrogen excretion was employed because it has been demonstrated that stimulation of Leydig-cell function by chorionic gonadotropin is accompanied by a prompt increase in estrogen excretion (5) and that the level of urinary estrogen excretion is a reliable indicator of Leydig-cell function (6, 7). *Urinary estrogens* were assayed in immature female rats as previously described (5), using uterine weight as the criterion of estrogenic activity. The results are expressed as the equivalent biologic activity of estradiol benzoate in micrograms per 24-hour urine specimen.

RESULTS

Plasma chorionic gonadotropin levels, determined while the patients were receiving chorionic gonadotropin in a dosage of *5,000 units intramuscularly three times weekly,* are listed in Table 2 and summarized in Table 3. In plasma samples obtained twenty-four hours after injection, chorionic gonadotropin levels averaged 30 units per 100 cc. Expressed in another manner, approximately 1,000 units could be accounted for in the estimated total plasma volume. From the second to the fourth day after injection, the amounts of hormone in the plasma remained fairly constant at levels which averaged about half of those observed in plasma specimens obtained twenty-four hours after injection. A marked decrease then occurred, and in plasma samples obtained more than seven days after the last injection,

TABLE 2. PLASMA CHORIONIC GONADOTROPIN LEVELS IN 10 MEN RECEIVING 5,000 INTERNATIONAL UNITS INTRAMUSCULARLY THREE TIMES WEEKLY

Patient	Time plasma obtained		Assay rats, uterine weight (mg.) (figures in parentheses indicate cc. of plasma administered to each rat)			Units CG per 100 cc.	Units CG in estimated* total plasma volume
	Days after onset of therapy	Hours since last injection					
M.B.	104	72	67 (½)†	139 (1½)†	141 (3)†	13	580
Hypogonadotropic eunuchoidism	136	72		70 (1½)†	104 (3)†	7	290
B.S.	before therapy	—			27 (6)	<2	<60
Hypogonadotropic eunuchoidism	13	24	55 (½)	118 (½)		40	1280
	26	72		31 (½)	83 (1)	10	320
J.S.	before therapy	—			27 (6)	<2	<80
Hypogonadotropic eunuchoidism	13	24	29 (½)	73 (½)		20	800
	26	72		37 (½)	107 (1)	15	600
M.S.	before therapy	—			28 (6)	<2	<80
Hypogonadotropic eunuchoidism	1	24	42 (½)	71 (½)		20	800
	2	48	33 (½)	69 (1½)	109 (3)	7	270
	14	24	37 (½)	79 (½)	128 (½)	40	1600
	27	72		45 (1½)†	110 (3)	7	270
	41	72	33 (½)	128 (1½)	163 (3)†	13	530
A.W.	before therapy	—			33 (6)	<2	<60
Hypogonadotropic eunuchoidism	14	72	42 (½)	122 (1½)		13	400
	21	72	40 (½)	56 (1)	128 (2)	10	300
	49	72		40 (1)	87 (2)	7	200
	77	72		28 (1)	67 (2)	5	150
	105	72		41 (2)	115 (4)	5	150
	112	168			26 (6)	<2	<60
G.Z.	94	72	33 (½)†	60 (½)	106 (1)	20	740
Panhypopituitarism	98	48	38 (½)	87 (½)		30	1110
	102	96	32 (½)	96 (½)	129 (1)	30	1110
	103	120		30 (1½)	98 (3)	5	185
	116	432			34 (6)	<2	<70
J.Z.	before therapy	—			25 (6)	<2	<80
Panhypopituitarism	1	24	30 (½)	103 (1½)		10	400
	28	72	40 (½)	142 (1½)		13	530
P.B.	1	24	34 (½)	113 (½)		40	800
Primary testicular failure	2	48		86 (½)	144 (1½)	30	600
	20	72		40 (½)	145 (1½)	20	400
	24	96		37 (½)	97 (1½)	10	200
	27	168			139 (6)†	>3	>60
R.P.	1	24	44 (½)	116 (½)		40	900
Primary testicular failure	14	72	34 (½)	60 (½)	138 (1)	20	450
	15	96		34 (½)	154 (1)	20	450
	17	144			61 (1½)	7	150
W.S.	before therapy	—			34 (6)	<2	<70
Normal		48	30 (1)	104 (2)		10	350
		72	57 (1)	97 (2)		10	350
		72		25 (2)	134 (6)	5	175
		240			44 (6)	<2	<70
		408			30 (6)	<2	<70

* Total plasma volume estimated as 5 per cent of body weight.

† Average for 2 rats; all other figures are averages for 3 or more rats.

TABLE 3. PLASMA CHORIONIC GONADOTROPIN LEVELS IN 10 MEN RECEIVING 5,000 INTERNATIONAL UNITS INTRAMUSCULARLY THREE TIMES WEEKLY

No. of hours after last injection plasma obtained	Units CG/100 cc. average—(range)	Units CG in estimated total plasma volume	No. of patients
24	30 (10–40)	940	7
48	19 (7–30)	580	4
72–96	13 (5–30)	450	10
120–168	4 (<2–7)	<150	4
More than 168	<2	<70	2

no hormonal activity could be detected. There was little cumulative effect from prolonged therapy; plasma levels during the first week or two of therapy were similar to those observed after several weeks or months of treatment.

In 3 patients receiving *2,500 units three times weekly*, plasma levels were

TABLE 4. PLASMA HORMONE LEVELS IN MEN RECEIVING VARIOUS DOSAGES OF CHORIONIC GONADOTROPIN

Patient	Chorionic gonadotropin therapy	Time plasma obtained		Assay rats, uterine weight (mg.) (figures in parentheses indicate cc. of plasma administered to each rat)				Units CG per 100 cc.	Units CG in estimated total plasma volume
		Days after starting therapy	Hours since last injection						
P.B.	5,000 units I.M. once weekly	14	168				66 (6)	2	33
		20	144			35 (3)	96 (6)	3	50
A.W.	5,000 units I.M. twice weekly	21	48			36 (1)	87 (2)	7	200
G.Z.	5,000 units I.M. daily	42	1	35 ($\frac{1}{10}$)	43 ($\frac{1}{2}$)	127 ($\frac{1}{2}$)	117 ($\frac{1}{2}$)	80	3,000
		50	24	29 ($\frac{1}{10}$)	74 ($\frac{1}{2}$)	118 ($\frac{1}{2}$)		80	3,000
B.S.	2,500 units I.M. 3 times weekly	12	72			40 (1)	85 (2)	7	210
		55	24		63 ($\frac{1}{2}$)	90 (1)		20	640
J.S.	2,500 units I.M. 3 times weekly	12	72			41 (2)	119 (4)	5	200
		55	24	36 ($\frac{1}{2}$)	100 (1)			15	600
M.S.	2,500 units I.M. 3 times weekly	28	72			49 (1$\frac{1}{2}$)	106 (3)	7	270
		42	72		32 ($\frac{1}{2}$)	79 (1$\frac{1}{2}$)	95 (3)	7	270
		54	24	43 ($\frac{1}{2}$)	100 ($\frac{1}{2}$)			30	1,200
	2,500 units in 30% polyvinylpyrollidone I.M. 3 times weekly	42	72			54 (1)	93 (2)	10	400
		49	240				97 (6)	3	130
		54	360				26 (6)	<2	<80
	5,000 units intravenously 3 times weekly	1	24		29 ($\frac{1}{2}$)	88 ($\frac{1}{2}$)	150 ($\frac{1}{2}$)	50	2,000
		2	48			34 ($\frac{1}{2}$)	81 ($\frac{1}{2}$)	25	1,000
		14	72	22 ($\frac{1}{10}$)	79 ($\frac{1}{2}$)	122 ($\frac{1}{2}$)	118 ($\frac{1}{2}$)	100	4,000
		22	168			53 ($\frac{1}{2}$)	95 ($\frac{1}{2}$)	30	1,200

about half those observed in patients receiving *5,000 units three times weekly* (Table 4). One patient received *5,000 units daily*, and his plasma levels were two to three times those observed in patients receiving *5,000 units three times weekly*. Another patient received *5,000 units intravenously three times weekly*. Strikingly high levels were observed in his plasma, and large amounts were still present seven days after the last injection. Plasma levels were determined in 1 of the 2 patients who received chorionic gonadotropin in polyvinylpyrollidone. The levels were similar to those observed after administration of the same dose of chorionic gonadotropin in aqueous solution, except that ten days after the last injection a small amount of hormone could be detected in the plasma.

Furthermore, there was no indication that polyvinylpyrollidone appreciably enhanced the activity of chorionic gonadotropin in the 2 hypogonadotropic eunuchoids (Table 5), since when polyvinylpyrollidone was substituted for water as the diluent, no significant change in estrogen excretion occurred and the subjective response, as judged by number of erections, was not altered.

TABLE 5. EFFECT OF POLYVINYLPYROLLIDONE ON CHORIONIC GONADOTROPIN ACTIVITY IN 2 HYPOGONADOTROPIC EUNUCHOIDS—USING URINARY ESTROGEN EXCRETION AS AN INDEX OF LEYDIG-CELL STIMULATION

Patient	Therapy	Urinary* estrogens, (micrograms per 24 hrs.)	No. of determinations
M.B.	Before treatment	0.2 (0.2–0.3)	4
	CG in water, 2,500 units 3 times weekly for 5 weeks	3.1 (2.3–3.5)	3
	CG in water, 1,250 units 3 times weekly for 5 weeks	1.8 (1.2–2.1)	3
	CG in 30% PVP, 1,250 units 3 times weekly for 8 weeks	2.2 (1.9–3.0)	3
M.S.	Before treatment	0.3 (0.3–0.3)	2
	CG in water, 5,000 units 3 times weekly for 4 weeks	1.0 (0.6–1.4)	3
	CG in water, 2,500 units 3 times weekly for 13 weeks	0.5 (0.3–0.6)	5
	CG in 30% PVP, 2,500 units 3 times weekly for 5 weeks	0.6 (0.6–0.6)	2

* Range for normal adult men: 0.7 to 1.3 micrograms per 24 hours.

DISCUSSION

Our observations that relatively high plasma hormone levels were maintained for several days after intramuscular injection is in agreement with the excretion studies of Bradbury and Brown (1) and Lloyd and co-workers (2), who observed that chorionic gonadotropin could be detected in the urine for several days following intramuscular injection. The observation that high plasma levels were maintained for several days after *intravenous* administration, would indicate that the important factor accounting for prolonged blood levels following intramuscular administration is slow disposal of the hormone, rather than delayed absorption. In fact, plasma levels were much higher seven days after intravenous injection than after intramuscular injection of chorionic gonadotropin, suggesting that some of the hormone may be destroyed at the site of intramuscular administration.

Since plasma levels of chorionic gonadotropin are maintained for several days following injection of aqueous solution of the hormone, it is not surprising that neither plasma levels nor therapeutic effectiveness of the hormone are altered when polyvinylpyrollidone is substituted for water as the diluent. Furthermore, urinary excretion rates are not markedly affected by the addition of materials which delay absorption from the site of injection (1,2). From the practical standpoint, it would appear that relatively constant plasma levels of chorionic gonadotropin can be obtained by administering the hormone twice weekly intramuscularly in aqueous solution. The observation that materials capable of prolonging absorption augment the activity of chorionic gonadotropin in rats, but not in human subjects, may be explained by assuming that the rat, unlike man, disposes of chorionic gonadotropin at a very rapid rate. Thus, prolonged absorption would allow more constant blood levels and, therefore, more effective hormonal action in the rat.

The plasma levels of chorionic gonadotropin in our patients approached those which have been observed during the second half of pregnancy. Our findings can be compared best with the findings in pregnant women reported by Jones, Delfs and Stran (8), since they used an assay method basically the same as that employed by us. If their values are divided by ten to account for the augmenting effect of plasma, the average plasma levels that they observed during the second half of pregnancy ranged from 30 to 100 international units per 100 cc. Our values for plasma obtained twenty-four hours after injection from men receiving 5,000 units three times weekly were at the lower limit of this range and the determinations in the patient receiving 5,000 units per day were well within this range.

Since values for the amounts of chorionic gonadotropin in the blood of pregnant women have been calculated without taking into account the augmenting properties of plasma, it is likely that most reported figures are much higher than the actual amounts. The effects of nonhormonal materials may also account for differences in conclusions concerning urinary excretion of chorionic gonadotropin. By measuring the amount excreted in the urine, Bradbury and Brown (1) were able to account for more than 50 per cent, and Lloyd *et al.* (2) were able to account for as much as 30 per cent of a single dose of chorionic gonadotropin administered intramuscularly to nonpregnant women. Johnson, Albert and Wilson (9), however, calculated that only 5 per cent of the hormone in the blood of pregnant women at the time of delivery was subsequently excreted in the urine. Although these divergent results could be due to differences in excretion by pregnant and nonpregnant individuals, it seems more likely that they are due to differences in the nonhormonal materials present in urine as compared to plasma. Moreover, the extent to which results are affected by nonhormonal materials may be expected to vary according to the type of assay method employed. With a rapid four-hour assay in which ovarian hyperemia was the endpoint used for detecting chorionic gonadotropin activity, Albert and Berkson (10) observed a depressing effect from large amounts of plasma. It may be that with the rapid methods of assay, augmenting substances delay absorption to the degree that only part of the hormone is absorbed by the time the procedure is completed.

SUMMARY AND CONCLUSIONS

Using a standard bioassay procedure (uterine weight of immature rats) and taking into account the fact that plasma produces a tenfold augmentation of chorionic gonadotropin activity in rats, a concentration of 0.03 international unit per cc. could be detected in the plasma of men receiving the hormone. In 10 men receiving 5,000 units intramuscularly three times weekly, a relatively high level of chorionic gonadotropin was maintained for three to four days after injection and then there was a rapid decrease. A high plasma level was also maintained for several days following *intravenous* administration, indicating that the important factor which maintains the blood level is slow disposal of the hormone. Neither the therapeutic effectiveness nor the plasma level of the hormone was appreciably altered when polyvinylpyrollidone was substituted for water as the diluent for intramuscularly administered chorionic gonadotropin. It is concluded that reasonably constant blood levels of chorionic gonadotropin, and therefore optimum therapeutic effects, may be obtained by administering an aqueous solution of the hormone twice weekly intramuscularly.

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RATE OF GROWTH OF AXILLARY HAIR AS A DIAGNOSTIC INDEX*

LAURANCE KINSELL, M.D.,† DOROTHY BRYANT AND FULLER ALBRIGHT, M.D.

The Department of Medicine, Harvard Medical School and the Medical Service of the Massachusetts General Hospital, Boston, Mass.

IN a report of patients with ovarian agenesis, Albright, Smith and Fraser (1) cited evidence that the growth of axillary and pubic hair in the male is under the hormonal control of the adrenal cortex and the Leydig cells of the testes, whereas in the female it is under the control of the adrenal cortex alone. The present study was undertaken to see whether the measurement of axillary hair growth by weight might be useful for the diagnosis and elucidation of endocrine disorders. The authors were particularly interested to determine whether a correlation exists between such a measurement and the urinary 17-ketosteroid excretion. All observations which follow relate to the *rate* of increase in hair mass, and not to linear growth.

METHODS

Both axillae are shaved at intervals of from two to six weeks. Patients are instructed not to use any powder or other local application. After a thorough washing of the axillae, shaving is carried out with a simple lather technique, the mixture of soap, lather and hair being washed from the razor into a beaker of hot water. This mixture is then filtered. The hair is washed free of any soap residue with repeated portions of hot water and collected at the apex of the filter paper. The excess filter paper is cut away; the remainder is dried in an oven or desiccator and weighed on an analytical balance before and after removal of the hair with a fine brush. It is essential that the weighing be carried out rapidly, to avoid uptake of moisture by the filter paper. All data are expressed as milligrams per axilla per week.

Urinary 17-ketosteroids and gonadotropins were determined by methods previously described (2) (3).

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Most of this work was carried out during the period 1941–1943. A preliminary draft of the paper, prepared while one of the authors (L.K.) was with the Navy on Samar, Philippine Islands, was lost en route to the Massachusetts General Hospital. After many revisions and delays, the work is finally reported in the present form.

† Research Fellow in Medicine and Pharmacology, Harvard Medical School, and Massachusetts General Hospital, 1941–1943. At present, Director, Institute for Metabolic Research, Highland Alameda County Hospital, Oakland 6, California.

TABLE 1. AXILLARY HAIR GROWTH AND URINARY 17-KETOSTEROID EXCRETION IN NORMAL MALES AND FEMALES

Subject	Age (yrs.)	Axillary hair				17-KS (mg./24 hrs.)	Axillary hair / 17-KS
		No. of assays	mg./axilla/wk.				
			High	Low	Av.		
				MALES			
KLI	29	2	6.3	5.5	5.9	17.5	$\frac{5.9}{17.5}=0.34$
KIN	34	10	12.4	5.9	8.6	13.4	$\frac{8.6}{13.4}=0.64$
REI	34	9	11.3	6.9	8.4	12.7	$\frac{8.4}{12.7}=0.66$
STA	32	2	9.2	9.2	9.2	16.0	$\frac{9.2}{16.0}=0.57$
LAW	30	4	16.2	11.3	14.1	8.7	$\frac{14.1}{8.7}=1.62$
CAS	25	3	10.4	8.6	9.3	19.2	$\frac{9.3}{19.2}=0.48$
SCH	25	7	12.5	7.4	9.1	9.7	$\frac{9.1}{9.7}=0.94$
BAL	26	4	14.7	9.7	12.2	16.7	$\frac{12.2}{16.7}=0.73$
				FEMALES			
DON	24	12	8.2	7.1	7.7	8.8	$\frac{7.7}{8.8}=0.87$
GOR	36	7	10.9	6.9	9.4	10.9	$\frac{9.4}{10.9}=0.86$
DON	25	2	15.7	10.0	12.9	6.2	$\frac{12.9}{6.2}=2.08$
BRY	24	13	8.7	5.6	6.8	7.5	$\frac{6.8}{7.5}=0.91$
TRE	23	7	7.6	5.5	6.1	10.6	$\frac{6.1}{10.6}=0.57$
MOO	32	8	6.0	4.7	5.5	11.8	$\frac{5.5}{11.8}=0.47$
NIC	30	10	11.8	7.9	9.7	9.6	$\frac{9.7}{9.6}=1.01$
ALB	28	8	9.0	6.0	7.5	6.4	$\frac{7.5}{6.4}=1.17$
MUR	21	3	14.0	11.5	12.9	5.3	$\frac{12.9}{5.3}=2.43$
LAW	26	8	9.1	7.6	8.2	4.1	$\frac{8.2}{4.1}=2.00$
COR	29	13	3.4	1.5	2.5	4.3	$\frac{2.5}{4.3}=0.58$
KIN	28	4	12.3	10.8	11.3	9.6	$\frac{11.3}{9.6}=1.18$

TABLE 1—*Continued*

STATISTICAL EVALUATION

	Axillary hair		17-Ketosteroids	
	Mean	S.E. of Mean	Mean	S.E. of Mean
Male	9.60	0.88	14.24	1.33
Female	8.39	0.88	7.92	0.77
Difference	*1.21*	*1.90*	*6.32*	*1.51*

AXILLARY HAIR GROWTH IN NORMAL MALES AND FEMALES

In Table 1 are shown the axillary hair and 17-ketosteroid values obtained in a group of normal males and females between the ages of 21 and 36 years. The mean value for axillary hair in females was 8.39 milligrams per axilla per week, with a standard deviation of ±0.88; the mean value in males was 9.60, with a standard deviation of ±0.88. There was no statistical correlation between the axillary hair growth and the urinary 17-ketosteroid excretion in these normal individuals. In observations which follow, however, it will be seen that, as a result of treatment or change in endocrine status, the axillary hair and 17-ketosteroid values often vary in the same direction.

RATE OF AXILLARY HAIR GROWTH

The majority of controls and patients studied were shaved at four-week intervals. For comparison, the values in terms of growth per week, obtained when shorter and longer intervals were used, are shown in Table 2. In people with normal rates of hair growth the results are essentially the same over an interval-range of two to eight or more weeks. Short intervals should not, however, be used in the study of individuals whose hair growth is abnormally slow, as the error under such circumstances may be high.

Seasonal variation

Figure 1 shows a continuous study extending over a period of eighteen months in a patient whose endocrine status changed little, if at all, during this time. The rate of growth of axillary hair reached a peak for both years during the months of June and July. Similar findings were noted in controls. This supports the observation that the beard appears to grow more luxuriantly during hot weather. The seasonal factor must be taken into account in the evaluation of axillary hair growth in relation to specific therapeutic procedures.

TABLE 2. RELATIONSHIP OF AXILLARY HAIR GROWTH TO LENGTH OF TIME BETWEEN SHAVINGS

Subject	Weeks															
	2	2½	3	3½	4	4½	5	5½	6	6½	7	7½	8	8½	9	9½	10
NORMAL MALES																	
KIN	8.0				*4** 9.0		9.9		9.0				7.3	9.0			6.5
REI					*6* 8.4		8.1								9.1		
GRE					3.5		*2* 3.5		*2* 3.5								
RAW					*4* 12.3		10.8						10.4				
LAW					*3* 13.6						15.6						
CAS					*2* 9.5		8.9										
SCH					*4* 9.1	9.1	8.4		10.1								
BAL					10.0	14.7										13.9	
NORMAL FEMALES																	
DON			*2* 7.4		*8* 7.7						8.1						8.0
GOR					*5* 9.8		10.2						6.9				
BRY					*11* 6.6		8.7						6.4				
TRE					*5* 6.2		6.0						5.6				
MOO	6.0				*7* 5.7						5.1						
NIC	11.4				*8* 9.4						10.3						
ALB					6.7		*2* 8.2		*3* 7.2		9.0		6.2				
MUR			11.5		*2* 13.7												
COR			2.7		*8* 2.4		*4* 2.6										
KIN					*2* 11.1												*2* 11.6

* The number of observations (of which the value shown is the mean) is indicated by the number in italics.

Effect of local application of methyltestosterone[1]

In Figure 2 are shown the findings in a male patient with panhypopituitarism, A.H. (M.G.H. #68854), who had no axillary or pubic hair. During the application of an ointment containing methyltestosterone (2 mg. per day) to the left axilla and of a control ointment to the right axilla, impressive growth of axillary hair occurred on the left side, eventually reach-

[1] The authors wish to thank the Schering Corporation for supplying the testosterone ointment.

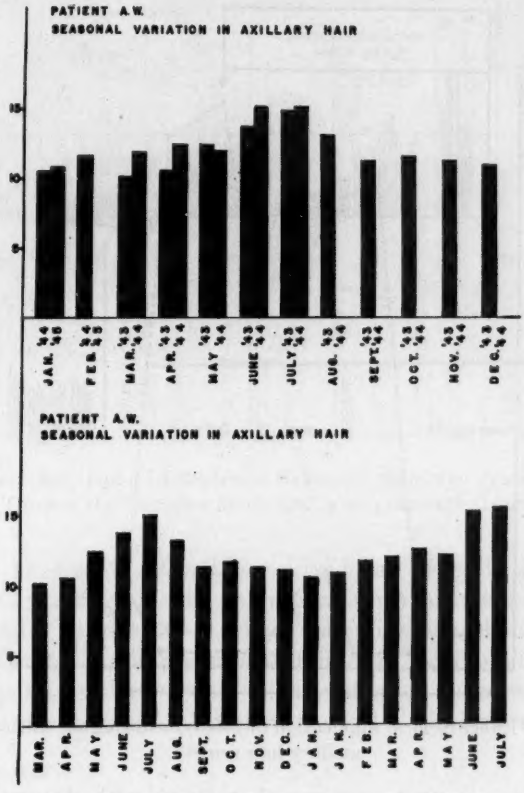

FIG. 1. Seasonal variations in axillary hair growth.

ing a nearly normal level. When the ointment was discontinued, hair growth did not stop until several months had elapsed. This observation has been made several times subsequently, as a part of another study now being carried out.

AXILLARY HAIR GROWTH AND 17-KETOSTEROID EXCRETION

In male and female patients with hypogonadism

In Figure 3 are shown the findings in a group of male and female patients with hypogonadal syndromes, representing a wide variety of endocrine diseases in an etiologic sense. All these patients manifested a significant degree of genital underdevelopment or atrophy.

The group of "male eunuchoids" includes those with levels of urinary follicle-stimulating hormone (FSH) which were decreased, normal, or

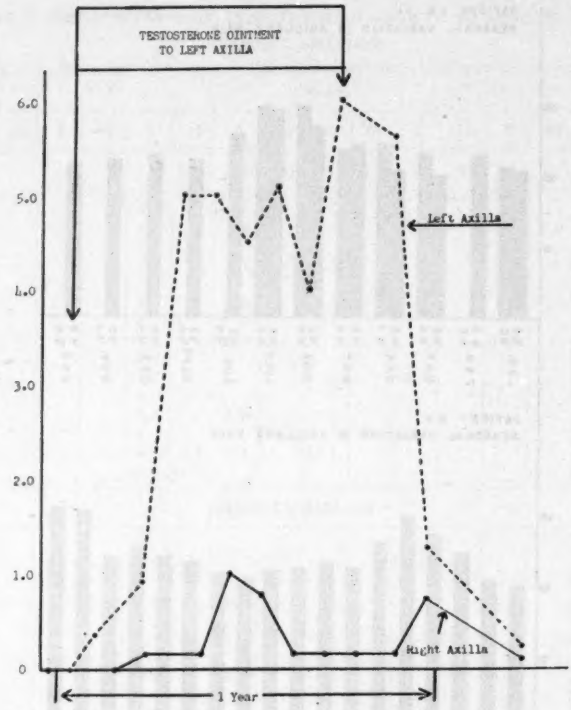

FIG. 2. Effect of local application of methyltestosterone ointment on axillary hair growth.

increased. It is proper to assume that those with decreased or absent FSH had primarily a pituitary pathologic condition, and that those with increased FSH had primarily a gonadal disorder. Regardless of etiology, when not receiving treatment all of these male eunuchoids had low rates of axillary hair growth; some of them, however, had 17-ketosteroid values well within the normal range.

Those men with high titers of urinary gonadotropin as part of the "*gynecomastia-small testis syndrome*" described by Klinefelter, Albright and Reifenstein (4), had more nearly normal axillary hair values than did male eunuchoids in general. This finding is compatible with the mild degree of hypoleydigism usually present in such patients.

The 4 patients in the *ovarian agenesis* group had recently had, or were receiving therapy. This probably accounts for the considerable variability in 17-ketosteroid excretion. The axillary hair values were low in all instances but there appears to have been some increase in response to estrogen therapy.

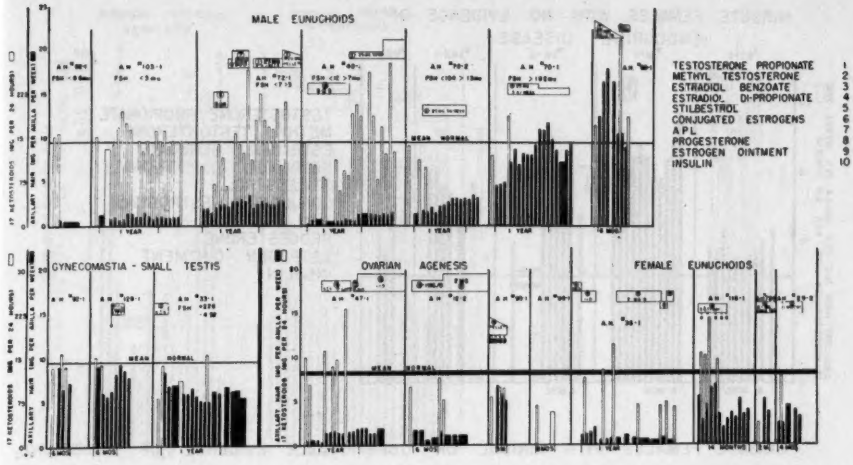

FIG. 3. Axillary hair and 17-ketosteroid values in male and female patients with hypogonadism. Three of the "female eunuchoids" were postsurgical menopause patients.

Under the heading of *"female eunuchoidism"* (Fig. 3) are included 3 patients (116-1; 72-2; 29-2) who had a premature menopause as the result of surgery, and 1 patient (36-1) whose endocrine disturbance was apparently due to a life-long and complete failure of FSH production. The 3 menopausal patients showed only slight reduction in growth of axillary hair, whereas the eunuchoidal patient showed a marked reduction.

In hirsute females

Hirsutism, obesity, and disturbances in menstrual function constitute a common clinical triad. Most patients with this syndrome have a familial history of hirsutism, but a definite endocrinopathy is seldom found. Their menstrual irregularities often respond to reduction in weight. Because of the possible existence of tumors of the adrenal cortex or gonads, all patients with hirsutism should be studied from an endocrine point of view. This applies particularly to patients whose heterosexual hypertrichosis does not appear at the onset of puberty.

In Figure 4 are shown values for axillary hair and 17-ketosteroid excretion, obtained in a group of patients with hirsutism. Some presented evidence of minor endocrine abnormalities, such as menstrual irregularities, which may or may not have been related to the hirsutism; others presented no evidence of endocrine disease. The data show that many hirsute females have a significant degree of acceleration in the rate of growth of axillary hair, and many have a somewhat increased urinary 17-ketosteroid

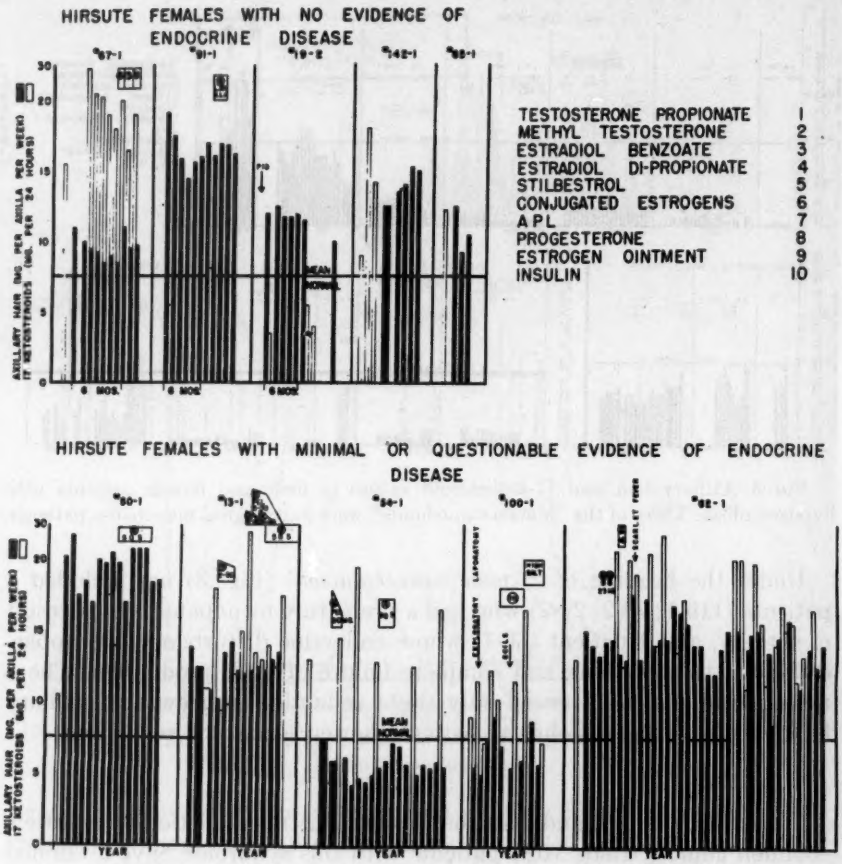

FIG. 4. Axillary hair and 17-ketosteroid values in female patients with "nonendocrine" hirsutism.

excretion. It follows that these two findings alone or in combination, are not sufficient to make the diagnosis of major endocrine pathology.

In patients with the adrenogenital syndrome and with Cushing's syndrome

In Figure 5 are shown data on 2 patients with the adrenogenital syndrome and 2 patients with Cushing's syndrome.

In Patient J.S., shortly after marriage, a slowly progressive hirsutism developed. At operation she was found to have a cancer of the right adrenal —and to be pregnant! The adrenal tumor was removed almost completely, and the patient aborted immediately following the operation. Over a period of many months there was local and metastatic regrowth of the

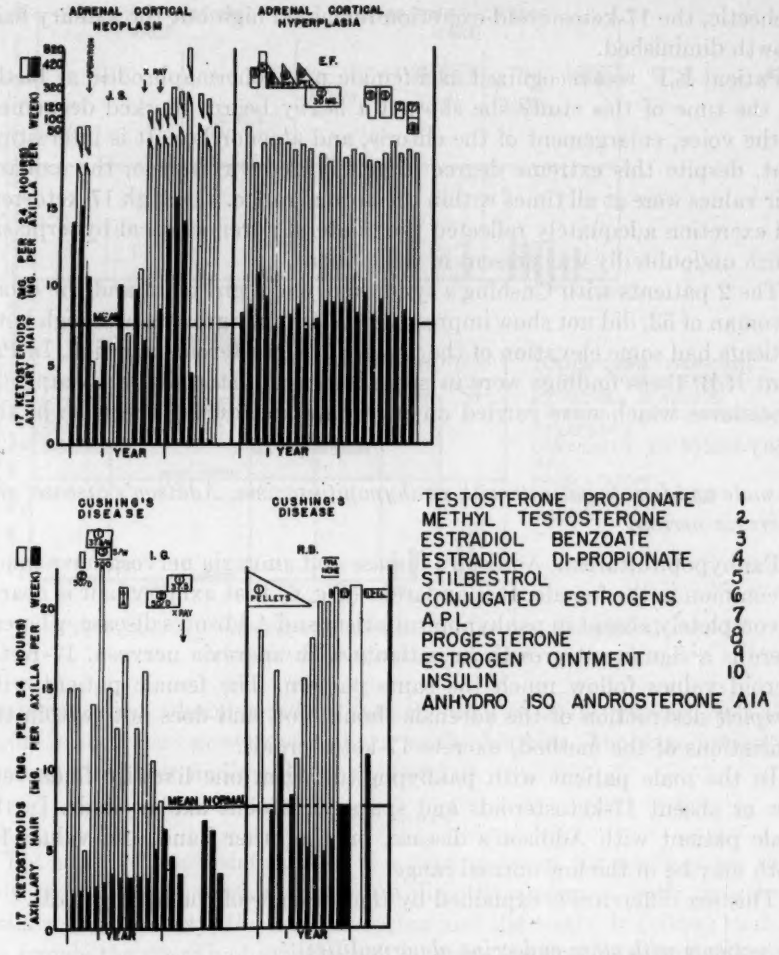

FIG. 5. Axillary hair and 17-ketosteroid values in the adrenogenital syndrome and Cushing's syndrome.

tumor. This was slowed briefly by x-ray therapy but the patient became worse and died. Throughout her entire illness, despite a 17-ketosteroid excretion level which at times was hundreds of milligrams per day, she presented no evidence of virilization other than hirsutism. Prior to operation there was a significant acceleration in axillary hair growth and an increase in urinary 17-ketosteroid excretion. Immediately following operation both values fell to subnormal levels, only to rise again after an interval of several months. During the terminal months of her life when she was

cachectic, the 17-ketosteroid excretion remained high but the axillary hair growth diminished.

Patient E.F. was recognized as a female pseudohermaphrodite at birth. At the time of this study she showed a heavy beard, marked deepening of the voice, enlargement of the clitoris, and amenorrhea. It is interesting that, despite this extreme degree of generalized virilization, the axillary hair values were at all times within the normal range. The high 17-ketosteroid excretion adequately reflected the bilateral adrenocortical hyperplasia which undoubtedly was present in this patient.

The 2 patients with Cushing's syndrome, one a girl of 13 and the other a woman of 53, did not show impressive axillary hair growth, although both patients had some elevation of the urinary 17-ketosteroid excretion. In Patient R.B. these findings were in some measure distorted by therapeutic procedures which were carried on preceding and during the time of the assay.

In male and female patients with panhypopituitarism, Addison's disease, and anorexia nervosa

Panhypopituitarism, Addison's disease and anorexia nervosa have much in common in the female. It is apparent (Fig. 6) that axillary hair is nearly or completely absent in panhypopituitarism and Addison's disease, whereas there is a significant growth in patients with anorexia nervosa. 17-Ketosteroid values follow much the same pattern. The female patient with *complete* destruction of the adrenals should not, and does not (within the limitations of the method) excrete 17-ketosteroids.

In the male patient with panhypopituitarism one likewise finds very low or absent 17-ketosteroids and sparse or absent axillary hair. In the male patient with Addison's disease, on the other hand, the values for both may be in the low normal range.

This sex difference is explained by the presence of the male gonads.

In patients with other endocrine abnormalities

In Figure 7 are shown composite data. It is apparent that the 17-ketosteroid and the axillary hair figures parallel each other to a considerable extent.

In response to administration of hormones

Many reports in the literature attest to the ability of testosterone preparations to raise the 17-ketosteroid excretion and to stimulate the growth of sex-linked hair in patients with panhypopituitarism and eunuchoidism. Methyltestosterone, although stimulating the growth of hair, lowers the 17-ketosteroid excretion. Albright *et al.* (1) have presented evidence that

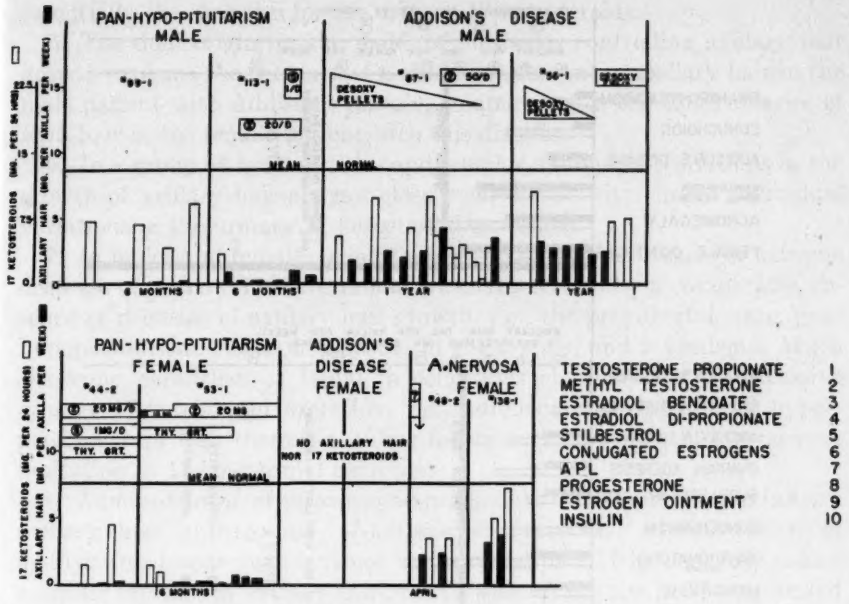

FIG. 6. Axillary hair and 17-ketosteroid values in panhypopituitarism, Addison's disease and anorexia nervosa.

estrogen, when administered to patients with ovarian agenesis, will also result in increased growth of axillary and pubic hair. The data presented here confirm these findings (Figs. 3, 5 and 6).

DISCUSSION

The authors' interpretation of the data rests on the assumption that only two of the endocrine glands are directly concerned with growth of axillary hair, namely, the adrenal cortex and the testis. It follows that in the female the growth of axillary hair is under the domination of only one gland, namely, the adrenal. Since the function of the adrenal cortex is dependent upon normal function of the anterior pituitary and thyroid, malfunction of any of four glands (adrenal cortex, testis, anterior pituitary or thyroid) may produce abnormality of axillary hair growth. Moreover, since the ovarian hormones significantly affect pituitary function, it is possible that the female gonad indirectly influences the growth of axillary hair.

SUMMARY AND CONCLUSIONS

1. A method for measuring the rate of growth of axillary hair is described.

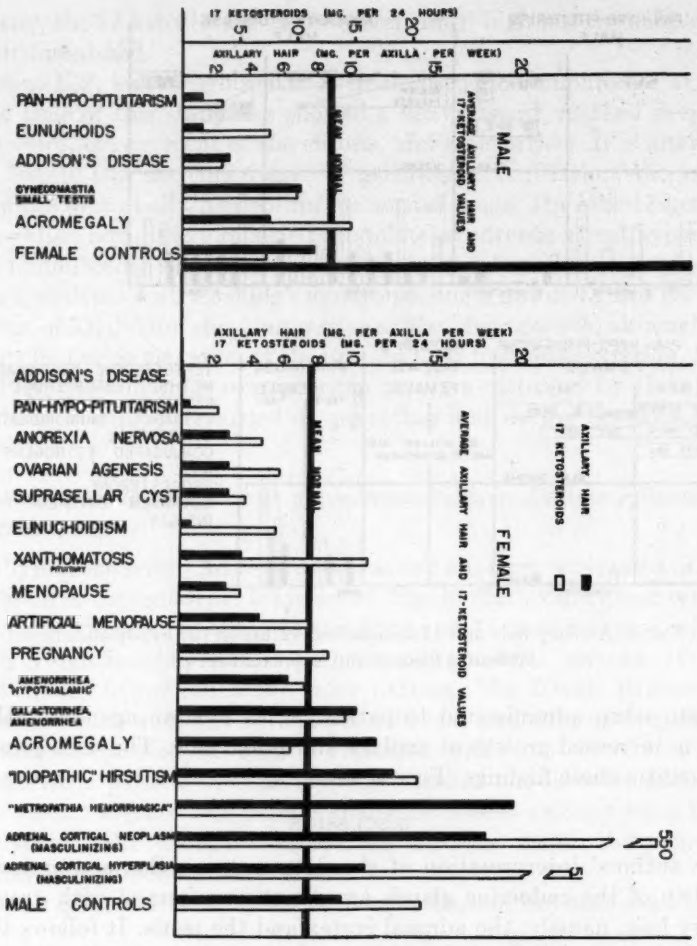

FIG. 7. Composite data. The values for "metropathia hemorrhagica" and "adrenal cortical neoplasm (masculinizing)" each come from 1 case only.

2. The rate of growth of axillary hair for any one individual is fairly constant, except for an increase during hot weather.

3. The mean rate of growth in normal young adult females is essentially equal to that found in normal young males.

4. In the female, the rate of growth of axillary hair is primarily dependent upon an as yet poorly defined hormone or group of hormones normally derived from the adrenal cortex; in the male it is dependent upon this hormone plus the androgenic hormone from the Leydig cells. This is

essentially the case also for the urinary 17-ketosteroids.

5. The dual origin, in the male, of hormones controlling axillary hair growth explains the presence of a moderate amount of axillary hair in the male patient with Addison's disease, compared with the usual absence of such hair in the female patient with this disorder.

6. In a group of normal males and females, individual variations in the growth of axillary hair are not closely correlated with similar individual variations in the urinary 17-ketosteroid excretion.

7. In males and females, conditions associated with absence or extreme decrease of urinary 17-ketosteroids are characterized by a comparable absence or decrease of axillary hair growth, *e.g.*, the prepubertal state, panhypopituitarism, Addison's disease in the female, and myxedema. Much the same parallelism is found in conditions characterized by excessive urinary 17-ketosteroid excretion, *e.g.*, adrenocortical tumor and hyperplasia, except that there is a ceiling for axillary hair growth, but no such limitation in 17-ketosteroid excretion.

8. Administration of testosterone propionate causes an increase in both axillary hair growth and 17-ketosteroid excretion; administration of methyltestosterone (which is not metabolized to a 17-ketosteroid) causes a similar increase in axillary hair growth without the rise in 17-ketosteroid excretion.

9. Local application of methyltestosterone ointment to one axilla of a patient with panhypopituitarism resulted in a marked stimulation of axillary hair growth on that side but not on the untreated side.

10. This study has failed to throw much light on the cause of "idiopathic hirsutism." Rapid growth of axillary hair is found in most of these patients.

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EFFECTS OF ACTH AND CORTISONE ON THE ADRENALS AND PITUITARIES OF CHILDREN WITH ACUTE LEUKEMIA*

BENJAMIN H. LANDING, M.D.† AND DAN FERIOZI, M.D.‡

The Tumor Therapy Service and the Department of Pathology, The Children's Medical Center, the Histochemistry Laboratory of the Children's Cancer Research Foundation, and the Department of Pathology, Harvard Medical School, Boston, Massachusetts

INTRODUCTION

THE introduction of compound E (cortisone) and adrenocorticotropic hormone (ACTH) as therapeutic agents has stimulated widespread interest in the relationship between the adrenal and the pituitary glands and in the control of their secretion. Both hypophysectomy and administration of cortisone have been shown to produce atrophy (decreased zone thickness and cell size) and to increase fat in the cells in the zona fasciculata and zona reticularis of the adrenal cortex (1, 2, 3). The effect of cortisone has been shown to be the result of a decrease in endogenous ACTH production (4–8). In general, administration of ACTH has been shown to increase cell size and zone thickness of the zona fasciculata and zona reticularis and to decrease cell fat and ascorbic acid in these zones (8–12). The relative independence of the zona glomerulosa has been demonstrated by its failure to show involutionary changes after hypophysectomy (1) or administration of cortisone (2, 3), by its failure to show proliferative or secretory changes after administration of ACTH or lyophilized anterior pituitary extract (5, 13), by its involutionary response to desoxycorticosterone (DCA) or sodium chloride, and by its secretory response to potassium chloride (14, 15). The production of hyalinized basophil cells (Crooke's change) in the anterior pituitary in man has been reported after ACTH and cortisone administration (16, 17).

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† Post-doctoral Research Fellow of the National Heart Institute. Present address: 2173 North Glebe Road, Arlington, Virginia.

‡ Present address: Department of Pathology, Children's Hospital Research Foundation, Cincinnati, Ohio.

The bulk of the work on the histologic aspects of the response of the adrenal cortex and anterior pituitary to these agents has been based on experimental animals, and relatively few studies on man have been reported (12, 18, 19). Therefore, it seemed desirable to review the material from children with acute leukemia treated with ACTH and cortisone, which was available in the Department of Pathology at The Children's Medical Center.

MATERIALS AND METHODS

All patients from whom material was studied were examined and treated by the Tumor Therapy Service of The Children's Medical Center. All had

TABLE 1. AGE AND SEX DISTRIBUTION OF PATIENTS WITH ACUTE LEUKEMIA

Age (yrs.)	Treated with ACTH or cortisone		Not treated with ACTH or cortisone	
	Male	Female	Male	Female
1	0	1	1	2
2	0	2	3	2
3	1	3	2	0
4	5	4	1	1
5	5	1	2	0
6	4	2	0	1
7	3	1	0	1
8	1	2	1	1
9	4	1	0	1
10	1	0	0	1
11–20	1	2	0	1
More than 20	1	0	0	0
Total	26	19	10	11
Grand total	45		21	

either acute leukemia or lymphosarcoma followed by an acute leukemic phase. Table 1 gives the age and sex distribution of the patients from whom material was examined, and Table 2 gives the length of time the patients who received either ACTH or cortisone were observed at the Tumor Therapy Clinic. Patients who had acute leukemia treated by folic-acid antagonists were used as controls because of the lack of adequate material from patients treated only with supportive measures, such as transfusions or antibiotics.

Adrenal and pituitary tissue removed at autopsy was placed in fixative as soon as possible after the death of a patient. Adrenal tissue was fixed in 10 per cent formalin and

TABLE 2. LENGTH OF TIME PATIENTS TREATED WITH ACTH OR CORTISONE WERE OBSERVED

Duration of observation (mos.)	No. of patients
0– 1	6
2	5
3– 4	2
5– 6	11
7–12	11
13–18	9
More than 18	1
Total	45

in Zenker-acetic acid solution; the pituitaries were fixed intact in 10 per cent formalin. The Zenker-fixed adrenal tissue was embedded in paraffin, sectioned at 5 microns, and stained with hematoxylin and eosin; the formalin-fixed adrenal tissue was cut on a freezing microtome at 15 microns, and stained with Sudan 4 (20) and by the Ashbel-Seligman technic for carbonyl groups (21). The pituitaries were embedded in paraffin, and a central section 5 microns thick was stained by Mallory's aniline blue technic (20). Sections of the adrenals were studied to determine the thickness of the three cortical zones, the size of the cells in each zone, the amount of fat in the cells, and the results of staining with Sudan 4 and the Ashbel-Seligman reaction. The relative thickness of the three adrenal cortical zones was recorded as "greater than," "not significantly different from," or "less than" average normal values. The average size of the cells in each of the three zones was evaluated in a similar way. The degree of staining of each zone with Sudan 4 was estimated as "none," "doubtful," "weak," "moderate," and "strong," and with the Ashbel-Seligman reaction as "none," "doubtful," "weak" and "moderate." The combined weight of the two adrenals from each patient was divided by the value for the combined weight of the glands for a patient of the same age and sex (22) and expressed as per cent of the "standard weight."

The relative number and size of the acidophil, basophil, and chromophobe cells of the anterior pituitary were recorded in the same manner, and the degree of granulation of the acidophil and basophil cells was also determined.

After the data had been obtained, the cases were divided into the following groups: 1) Controls—patients who received only folic-acid antagonist therapy; 2) patients who died while being treated with ACTH; 3) patients who died within two months after treatment with ACTH; 4) patients who died while being treated with cortisone; 5) patients who died within two months after treatment with cortisone; 6) patients who died while being treated with both ACTH and cortisone; 7) patients who died while being treated with cortisone after having received ACTH; and 8) patients who died while being treated with ACTH after having received cortisone. Within each group, for each variable being studied (for example, the size of the cells of the zona fasciculata), the number of instances in which the value

for the particular variable fell into each of the various classes was determined, and these values were converted into percentages. These percentages were tabulated, and the behavior of the different variables in the varying situations studied was determined (Tables 3 to 6).

TABLE 3. EFFECT OF ACTH ON WEIGHT OF ADRENALS FROM CHILDREN WITH ACUTE LEUKEMIA

Group	No. of cases in which adrenals exam'd.	Avge. total dose (mg.)	Avge. duration of interval (days)	Avge. weight (% of standard)
1. Leukemic controls (folic-acid antagonist treatment)	21			184
2. ACTH	9	603		219
3. After ACTH	9	599	31.5	171
4. Cortisone	9	1,294		179
5. After cortisone	7	1,767	28.5	176
6. ACTH and cortisone	2	Cortisone, 213; ACTH, 114		369
7. ACTH followed by cortisone	2	ACTH, 850; cortisone, 728		138
8. Cortisone followed by ACTH	7	Cortisone, 1,865; ACTH, 302		189
Total	66			

RESULTS

Results of the studies in each group appear in Tables 3 to 6. Because of the number of suggestive, but not conclusive observations, it seems desir-

TABLE 4. RESULTS OF SUDAN 4 STAIN ON ADRENALS FROM CHILDREN WITH ACUTE LEUKEMIA TREATED WITH ACTH AND CORTISONE

Group	Zona glomerulosa					Zona fasciculata					Zona reticularis				
	None	Doubtful	Weak	Moderate	Strong	None	Doubtful	Weak	Moderate	Strong	None	Doubtful	Weak	Moderate	Strong
1	10	35	35	10	10	35	25	30	5	5	10	50	30	5	10
2	11	44	44	0	0	55	22	22	0	0	44	37	22	0	0
3	11	33	44	0	11	22	11	44	0	22	22	22	33	0	33
4	11	11	44	0	33	22	0	55	0	22	11	22	44	0	22
5	37	12	50	0	0	37	25	37	0	0	50	12	37	0	0
6	0	0	0	100	0	0	0	50	50	0	50	0	50	0	0
7	50	0	50	0	0	0	50	50	0	0	0	50	50	0	0
8	72	28	0	0	0	43	28	14	14	0	57	43	0	0	0

TABLE 5. RESULTS OF ASHBEL-SELIGMAN REACTION ON ADRENALS FROM CHILDREN WITH ACUTE LEUKEMIA TREATED WITH ACTH AND CORTISONE

Group	Zona glomerulosa				Zona fasciculata				Zona reticularis			
	None	Doubtful	Weak	Moderate	None	Doubtful	Weak	Moderate	None	Doubtful	Weak	Moderate
1	70	20	10	0	60	15	25	0	65	10	25	0
2	44	33	22	0	33	33	33	0	33	33	33	0
3	44	0	44	11	33	22	33	11	22	33	33	11
4	44	33	22	0	44	0	55	0	22	33	33	11
5	25	25	37	12	25	25	50	0	25	25	50	0
6	50	0	50	0	50	0	50	0	50	0	50	0
7	50	0	50	0	50	0	50	0	50	0	50	0
8	57	28	0	14	57	14	28	0	72	14	14	0

TABLE 6. EFFECTS OF ACTH AND CORTISONE ON ANTERIOR PITUITARIES OF CHILDREN WITH ACUTE LEUKEMIA

Group	No. of pituitaries exam'd.	*Acidophils*									*Basophils*									*Chromophobes*					
		Cell size			Cell count			Granules			Cell size			Cell count			Granules			Cell size			Cell count		
		small	normal	large	low	normal	high	low	normal	high	small	normal	large	low	normal	high	low	normal	high	small	normal	large	low	normal	high
1	11	27	63	9	63	27	9	0	72	27	18	54	27	18	9	72	27	27	45	27	45	27	27	36	36
2	8	0	75	25	37	37	25	0	50	50	37	12	50	25	25	50	62	0	37	37	12	50	25	25	50
3	9	11	88	0	22	77	0	0	77	22	11	44	44	22	44	33	50	50	0	11	44	44	11	66	22
4	9	22	55	22	11	55	33	22	33	44	22	44	33	44	22	33	44	22	33	22	55	22	33	55	11
5	6	17	50	33	17	66	17	0	50	50	0	50	50	0	50	50	66	17	17	33	16	50	0	83	17
6	2	0	100	0	0	100	0	0	100	0	0	0	100	0	50	50	50	50	0	0	50	50	0	50	50
7	1	0	100	0	0	100	0	0	100	0	0	0	100	0	100	0	100	0	0	0	100	0	0	100	0
8	6	66	17	17	50	17	33	0	17	83	17	17	66	33	33	33	50	17	33	0	40	60	20	40	40

able to include some explanatory material, generally reserved for the discussion, in this section.

Group 1.—The control adrenals (from patients treated with folic-acid antagonists) had a normal range of values for zone thickness and cell size in the sections stained with hematoxylin and eosin; the apparent fat content, estimated from the degree of vacuolation of the cytoplasm, was relatively low in the zonae fasciculata and reticularis. In Sudan 4 preparations, there was a low fat content in all three zones; the Ashbel-Seligman reaction was weak in all three zones, also. The average combined weight of the adrenals, in terms of per cent of standard, was large, but no adequate data are available on normal adrenal weights in this age group and population. The average number of acidophil cells in the anterior pituitary appeared to be low, and the number of basophils high, in the folic acid antagonist-treated controls.

Group 2.—In patients who died while being treated with ACTH, the average combined weight of the adrenal glands was increased. The zona fasciculata and reticularis were increased in thickness, their cells were larger than normal, and their fat content decreased. The zona fasciculata showed a questionably greater decrease in fat content than the zona reticularis, suggesting that it is somewhat more sensitive to ACTH than is the reticularis. The zona glomerulosa showed no apparent change in the preparations stained with hematoxylin and eosin or Sudan 4. The degree of staining with the Ashbel-Seligman procedure rose slightly in all three zones. In the anterior pituitary, the acidophil cells showed no apparent differences from the folic acid antagonist-treated controls. The basophils showed an increase in average size, degranulation, and a questionable decrease in numbers, whereas the chromophobe cells showed questionable increases in size and number.

Group 3.—In patients dying after having received a course of ACTH therapy (the average interval between the end of ACTH therapy and death was 31.5 days), the adrenals in sections stained with hematoxylin and eosin showed a return to normal appearance. In sections stained with Sudan 4, the fat content of the two inner zones rose to the normal range, and perhaps slightly above it, and the average combined weight of the adrenals fell to slightly below the control value. These two phenomena suggest that the period of depressed endogenous ACTH production persists for a somewhat longer time after cessation of administration of ACTH than is apparent clinically. The Ashbel-Seligman reaction of the adrenals, and the degranulation of the basophil cells of the anterior pituitary showed no regression from the effects produced by ACTH.

Group 4.—In hematoxylin and eosin stained sections of adrenals from patients who died while being treated with cortisone, the zona glomerulosa

showed no apparent difference from the control values, whereas in the two inner zones, the fat content rose. In sections stained with Sudan 4, the fat increased in all three zones; the Ashbel-Seligman reaction also increased in all three zones, to about the same degree as with ACTH. The average combined weight of the adrenals was lower than that of the folic acid antagonist-treated controls, but not significantly so. In the anterior pituitaries, the acidophil cells showed an apparent increase in number, and in the degree of cytoplasmic granulation, whereas the basophil cells showed degranulation and a decrease in number. The chromophobes showed no apparent change.

Group 5.—Among patients who died after treatment with cortisone, the average interval between termination of treatment and death was 28.5 days. In the hematoxylin and eosin stained sections of the adrenals, the zona glomerulosa did not differ from that of the controls, or from the condition seen during cortisone treatment, but the two inner zones showed an increase in thickness and cell size and a decrease in cell fat to, and perhaps slightly beyond, control values. In the Sudan 4 preparations the fat content fell to, or slightly below, the control values. These changes suggested a mild degree of ACTH effect, perhaps only as regards secretory activity, since the weight of the glands did not change significantly from the values seen during cortisone therapy, and the Ashbel-Seligman reaction in the adrenals and the findings in the anterior pituitary did not return to the control values in the interval studied.

Group 6.—In patients who died while being treated with both ACTH and cortisone, the hematoxylin and eosin stained sections of the adrenals showed no significant differences from the control values, suggesting that these two drugs may, to some extent, cancel each other's microscopic effects on human adrenals. However, in the Sudan 4 preparations the fat content rose, as is seen during cortisone therapy, and the average combined adrenal weight rose, as is seen with ACTH therapy, suggesting that the various effects of these agents on the adrenals can be dissociated. Results of the Ashbel-Seligman reaction on the adrenals did not differ from control values. The anterior pituitaries showed a questionable increase in the number of eosinophils, and increased size and degranulation of the basophils, more suggestive of cortisone than of ACTH effect. However, the number of patients in this group is too small for the values to have significance; this is true also of the following group.

Group 7.—In those patients who died while being treated with cortisone after having received ACTH, sections of the adrenals stained with hematoxylin and eosin, Sudan 4, and the Ashbel-Seligman reaction did not differ from those of the controls, whereas the weights of the glands decreased, indicating that a cortisone effect had occurred. The anterior pituitaries also

showed changes suggestive of those seen after cortisone therapy.

Group 8.—In contrast to group 7, in patients who died while being treated with ACTH after having received cortisone, the hematoxylin and eosin stained preparations of the adrenals showed a definite decrease in fat content in the zona glomerulosa, as well as in the two inner zones, which showed the increase in thickness and cell size seen with ACTH therapy alone. The decrease in fat in all three zones was also shown by the Sudan 4 preparations. Results of the Ashbel-Seligman reaction did not differ from the control values. The average combined weight of the adrenals did not increase significantly, again suggesting some degree of antagonism between the two agents. In the anterior pituitaries, the acidophils showed a questionable decrease in number, and the basophils showed an increase in size and degranulation, and a questionable decrease in number, suggesting the change produced by ACTH alone.

DISCUSSION

The general effects of ACTH (in the doses used) on the adrenals of children with acute leukemia were an increase in zone thickness and cell size and a decrease in cell fat in the zona fasciculata and reticularis, but no significant change in the zona glomerulosa. Perhaps the zona fasciculata responded more than the reticularis. The Ashbel-Seligman "ketosteroid" reaction increased slightly in all three zones, and in the anterior pituitaries, the basophil cells increased in size, showed degranulation, and possibly decreased in number. In general, these changes are in accord with those reported in the literature for both humans and experimental animals. After administration of ACTH, the microscopic appearance of the adrenals returned to normal within two months, and the fat content rose to slightly more than the control values, while the weight fell to slightly less than control values. Presumably, this was due to the fact that endogenous ACTH production does not return to pre-therapy values during this time, so that after treatment with ACTH, the adrenals undergo, to a mild degree, the changes seen during cortisone therapy. The failure of the changes in the anterior pituitary to return to control values supports this interpretation.

After cortisone therapy, the adrenals showed a decrease in weight, zone thickness, and cell size in the two inner zones, with a rise in stainable fat in all three zones, and the basophils of the anterior pituitary showed degranulation similar to that seen with ACTH. These changes also are in accord with those reported in the literature for both humans and animals. However, the increase in fat in the zona glomerulosa suggests either that the glomerulosa is in part responsive to ACTH, or that cortisone has some direct effect on this zone. Within two months after cessation of cortisone therapy, the microscopic findings suggested increased activity of the zona

fasciculata and reticularis, but the weight of the glands did not increase as it does with administration of ACTH. These observations suggest that cortisone depression, either of endogenous ACTH production or merely of the cell-proliferative property of ACTH, persists for some time after cessation of cortisone therapy. It is not known whether small amounts of ACTH can stimulate cortical cell secretion without inducing cell growth and proliferation, or whether, under certain conditions, cortisone is able to block the latter without blocking the former. This problem merits further study. Perhaps paradoxically, when cortisone and ACTH were administered at the same time, both the fat content of the three zones and the weight increased. This observation, although not reliable because it was based on only 2 patients, again suggests that cortisone is able to produce dissociation of the various responses of the normal adrenal cortex to ACTH, and indicates that cortisone may have a direct effect on the adrenal cortex over and above its ability to inhibit endogenous ACTH production.

When treatment with ACTH was followed by administration of cortisone, the adrenal and pituitary showed essentially the same findings as after cessation of ACTH therapy, probably indicating that in the presence of ACTH-induced depression of endogenous ACTH secretion, cortisone can have little additional effect. However, when cortisone was followed by ACTH, the zona glomerulosa showed evidence of stimulation, with a definite decrease in fat content, similar to that seen in the two inner zones. This indicates not only that previous exposure to cortisone does not block the secretory response of the adrenal cortex to ACTH, but that it may stimulate this response, at least for the zona glomerulosa; the failure of the adrenal weights to increase as much as during administration of ACTH alone may suggest, as did the post-cortisone data, that previous exposure to cortisone inhibits the proliferative response of the adrenal cortex to ACTH. Probably of greatest significance, however, is simply the observation that under certain conditions—in this case, previous administration of cortisone—the human zona glomerulosa apparently responds to ACTH. This may occur also in patients with the nephrotic syndrome, as described in another paper (23).

No definite conclusion can be drawn from these studies as to the significance of the Ashbel-Seligman carbonyl reaction. The fact that the reaction increased after administration of ACTH and cortisone indicates that this procedure is not simply a fat stain. This and the fact that the reaction continued at an elevated level during the period in which the fat content returned to normal after cessation of administration of either of these drugs, suggest that there is no close relation between this reaction and adrenocortical activity.

Several factors affecting the validity of the conclusions drawn from this

study should be mentioned. The relatively small number of cases in each of the various groups prevents satisfactory statistical analysis, so that most of the findings are presented as suggestions rather than as demonstrated facts. A rather wide degree of variation in the histologic appearance of the adrenals in the various groups, within the framework of a general tendency, was clearly present. Whether this simply reflects the obvious inability to take into account each patient's endogenous ACTH secretion and its fluctuations, or whether it indicates that other factors (such as the serum cholesterol level) also affect the histologic appearance of the adrenals of seriously ill patients treated with these drugs, cannot be stated. No method of attempting to group patients with possibly similar degrees of endogenous ACTH production, by estimating in some way the degree of illness (using fever, weight loss, the impressions of the patient or observers, or other factors as criteria) seemed practicable, and such a procedure would have had the disadvantage of subdividing still further the small groups of patients.

SUMMARY AND CONCLUSIONS

The effects of ACTH and cortisone, administered singly, in combination, and in sequence, on the adrenals and anterior pituitaries of children with acute leukemia are reported.

ACTH caused an increase in adrenal weight, an increase in thickness and cell size of the zona fasciculata and zona reticularis, and a decrease in intracellular fat in these zones. The zona glomerulosa showed no significant changes. The basophils of the anterior pituitary increased in size and showed degranulation. After cessation of ACTH therapy, the adrenal changes regressed, and changes suggestive of depression of endogenous ACTH production were seen; the anterior pituitary changes did not regress in the interval studied.

Cortisone caused a slight decrease in adrenal weight, a decrease in thickness and cell size of the zona fasciculata and zona reticularis, and an increase in cell fat in all three zones, suggesting that the zona glomerulosa is responsive to ACTH, or that cortisone has a direct effect on the adrenal. The anterior pituitaries showed an increase in size and degranulation of the basophils, and a questionable increase in the number of acidophils. After cessation of cortisone therapy, the changes in the adrenals regressed, and evidences of increased secretory activity of the zona fasciculata and zona reticularis were observed, suggesting a direct effect of cortisone on the sensitivity of these zones to endogenous ACTH, since the anterior pituitary changes did not regress in the period studied.

Combined ACTH and cortisone therapy caused an increase in adrenal weight similar to that produced by ACTH, but an increase in cell fat similar to that produced by cortisone. This alteration of the morphologic re-

sponse to ACTH suggests that cortisone, by direct action on the adrenal, can dissociate the various normal cortical responses to ACTH. The anterior pituitaries in this situation showed changes more suggestive of cortisone than of ACTH effect.

ACTH followed by cortisone produced adrenal and pituitary changes suggestive of a simple cortisone effect. Cortisone followed by ACTH produced adrenal and pituitary changes similar to those produced by ACTH alone. In addition, there were secretory changes in the zona glomerulosa, suggesting that, under certain conditions, the human zona glomerulosa is responsive to ACTH.

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THE NEUTRAL STEROIDS OF HUMAN URINE: THE CORTICOSTEROID FRACTIONS

S. L. TOMPSETT AND D. C. SMITH

The Biochemical Laboratory, Northern General Hospital, Edinburgh, Scotland

IN PREVIOUS communications, one of us (S.L.T.) has reported the results of investigations concerning components of the so-called neutral steroid fraction of human urine (1, 2, 3). These studies were concerned with the significance of the nonketonic alcohols and 20-ketosteroids in this fraction. The extracts employed were prepared by the standard procedure used in the preparation of urine for the determination of neutral 17-ketosteroids. Urine was boiled with dilute mineral acid, cooled and then extracted with an organic solvent. The extract, after washing with aqueous alkali and water, was evaporated to dryness.

More recently, one of us (S.L.T.) has shown that these extracts react with periodic acid to produce formaldehyde (4)—a characteristic of the adrenocortical steroids related to corticosterone and cortisone. It was found that the 17-desoxycorticoids, desoxycorticosterone and corticosterone, were stable to the effect of hot dilute mineral acids, to the degree that their ability to react with periodic acid to produce formaldehyde was unimpaired by such treatment. Other structural changes, however, could have occurred which might affect biologic activity. This fraction has been designated as the corticosterone-like substances. It has now been decided to designate this fraction with the less specific term, acid-stable formaldehydogenic substances (ASFS), since it is now believed that part of the metabolites of the 17-hydroxycorticoids may be included as well as those of the 17-desoxycorticoids. In the normal adult, excretion of this fraction was found to amount to 4.5 to 7.5 mg./diem. The excretion of this fraction approached zero in hypoadrenalism and was markedly elevated in hyperadrenalism. Marked increases were observed following the administration of ACTH or desoxycorticosterone acetate. Stewart, Robson and Tompsett (5) have observed marked increases in the excretion of this fraction as the result of surgical trauma.

The reaction between periodic acid and the corticoids to produce formaldehyde is not specific. Many other substances that occur in biologic materials react likewise [Tompsett and Smith (6, 7)]. It is believed however that the initial processes do achieve a reasonable degree of specificity.

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The original procedure has been modified with the intention of increasing the degree of specificity. (A description is given in a subsequent section.) The main change has been to allow the urinary extract and periodic acid to react at room temperature, as in the procedure of Daughaday, Jaffe and Williams (8). Results obtained by the modified procedure are 15 to 20 per cent lower than by the original technique.

Recently Norymberski, Stubbs and West (9) have described a method for the measurement of a fraction in urine, referred to as the 17-ketogenic steroids. This value represents the increase in the level of 17-ketosteroids as a result of the treatment of urine in the cold with sodium bismuthate. It has been shown that 17-hydroxycorticoids such as compounds E and F, and also the 17-hydroxysteroid, pregnanetriol, react thus. The 17-desoxycorticoids are not included. According to these authors, the excretion of this fraction in the normal adult amounts to 4.6 to 19.2 mg./diem.

In this investigation, which is of a preliminary nature, 17-ketosteroids, ASFS and 17-ketogenic steroids have been measured in the urine in a number of selected cases. It is believed that such compounds should include a large proportion of the metabolites of the adrenocortical steroids that occur in urine. The object of this investigation was primarily to determine whether the quality as well as the quantity of the adrenocortical secretion could vary as the result of disease processes. It is believed that our results suggest such a hypothesis.

METHODS

The methods which have been employed are:

1. 17-Ketosteroids [Medical Research Council (10)]
2. 17-Ketogenic steroids [Norymberski *et al.* (9)]
3. Acid-stable formaldehydogenic substances (ASFS)—the following modification of the method of Tompsett (4):

An extract was prepared from 100 ml. of urine, according to the original instructions. The dried extract was dissolved in 1 ml. of ethanol, 5 ml. of periodate reagent (0.01M potassium periodate in 0.15M sulphuric acid) added, and the mixture allowed to stand twenty-four hours at room temperature. At the end of this period, the mixture was transferred to a distillation flask attached to a water-cooled condenser and after the addition of 0.5 Gm. of solid stannous chloride and 0.5 ml. of concentrated sulphuric acid, the volume was made up to 15 ml. with water. The mixture was heated to boiling and the distillate collected in 1 ml. of aqueous 1 per cent sodium sulphite solution until a total volume of 10 ml. was obtained. A complete blank was carried out at the same time. Formaldehyde was determined in 1 ml. of the distillate, as described in the original paper. Readings were made against the blank and results expressed in terms of desoxycorticosterone.

RESULTS

Results are shown in the Table 1.

TABLE 1. THE URINARY EXCRETION OF NEUTRAL STEROIDS IN VARIOUS CONDITIONS

17-KS = 17-ketosteroids, mg./diem.
17-KGS = 17-ketogenic steroids, mg./diem.
ASFS = acid-stable formaldehydogenic substances, mg./diem.

Age/Sex	Condition	17-KS	17-KGS	ASFS
	A. General—I			
39/M	Normal	9.7	7.8	4.0
35/M	Normal	9.0	11.1	4.3
67/M	Normal	14.0	7.0	6.1
46/M	Normal	6.3	9.0	3.7
48/F	Normal	7.5	9.7	4.1
28/F	Normal	8.5	7.2	4.0
	II			
19/M	Recurrent renal calculi	3.3	59.8	4.1
29/M	Recurrent renal calculi	5.6	37.2	4.0
77/F	Postop. hypotension	2.3	18.8	2.3
71/M	Carcinoma of prostate	3.4	30.2	3.4
74/M	Carcinoma of prostate	7.6	26.2	5.2
	III			
58/F	Recurrent renal calculi	4.1	2.1	9.7
51/F	Recurrent renal calculi	3.8	7.2	8.8
30/F	Recurrent renal calculi	9.5	3.7	7.8
	B. Effect of the administration of ACTH			
8/M	Nephrotic syndrome			
	Before ACTH	4.4	4.6	1.8
	ACTH	11.8	10.1	4.0
36/M	Ankylosing spondylitis			
	Before ACTH	7.5	13.4	2.3
	ACTH	9.8	20.1	5.2
41/M	Ankylosing spondylitis			
	Before ACTH	8.9	13.9	4.0
	ACTH	12.2	27.8	7.4
23/M	Acute porphyria			
	Before ACTH	15.0	75.0	35.2
	ACTH	35.0	141.0	68.0
6/F	Nephrotic syndrome			
	Before ACTH	1.6	0.0	1.7
	ACTH	15.5	2.4	31.7
77/F	Thrombocytopenic purpura			
	Before ACTH	2.5	3.0	1.1
	ACTH	5.4	27.3	9.4

TABLE 1 (*Continued*)

Age/Sex	Condition	17-KS	17-KGS	ASFS
	C. Effect of a surgical operation			
51/M	Before op.	6.7	19.2	3.0
	1st day after op.	9.5	20.5	14.0
	2nd day after op.	9.5	30.5	19.0
11/M	Before op.	5.1	11.6	6.8
	1st day after op.	8.5	5.1	16.2
	2nd day after op.	4.2	6.2	10.7
	3rd day after op.	2.7	6.5	6.3
13/F	Before op.	2.9	1.8	1.9
	1st day after op.	1.2	1.6	4.1
	2nd day after op.	2.1	2.7	5.8
	3rd day after op.	3.2	3.2	7.8
	D. Effect of severe infection			
7/M	On admission	8.1	2.8	8.8
	Convalescence	1.7	3.9	2.1

In the normal adult, the 17-KGS fraction is higher than the ASFS fraction. The effect of the administration of ACTH was to produce a general increase in all fractions except in 1 case. In this patient, a female child with the nephrotic syndrome, although there were marked increases in the excretion of the 17-KS and ASFS fractions, the administration of ACTH had very little effect on the excretion of the 17-KGS fraction. Similar results were noted in a case of acute infection. Surgical trauma produced some wide differences in effect. In an adult, there was a general increase in all fractions—rather similar to that expected from the administration of ACTH. In a male child, there was a marked increase in the excretion of the 17-KS and ASFS fractions but a decrease in the excretion of the 17-KGS fraction. In a female child, there was an increase in all fractions, the most marked change appearing in the ASFS fraction. The unusual delay of the response in this case is believed to have been due to the effect of a previous and recent trauma. Sections A, II and III, of Table 1 represent determinations on random specimens of urine. In section II a marked increase in the excretion of the 17-KGS fraction will be noted, without any corresponding changes in the excretion of the other fractions. In section III a marked increase in the excretion of the ASFS fraction will be noted, without any corresponding change in the excretion of the other fractions.

DISCUSSION

The results reported in this paper suggest that the nature of the adreno-

cortical secretion in man can vary not only in quantity but in quality—possible factors being age and the nature of the stimulus. Although this conception merits further investigation, some caution is required, since many factors are involved, some of which are not immediately capable of elucidation in view of the present state of our knowledge of the subject. The assumption is made that these fractions are derived entirely from the adrenal cortex. It is still necessary to show conclusively that substances derived from tissues other than the adrenal cortex, or even the diet, are not included in such quantity as to influence unduly the validity of the final results. The influence of the kidney must be considered. The kidney, especially as the result of hormonal stimulus, is a discriminating organ and its effect on the urinary excretion of steroidal end-products is as yet an unexplored subject. At present we do not know with any degree of certainty whether the major part of the adrenocortical output is excreted by the kidney or by some other route.

The exact nature of the adrenocortical secretion in man has still to be elucidated. Recent work by Romanoff and associates (11) suggests that the metabolites present in urine are many, but few have been chemically defined. The nature of the corticosteroids present in human blood appears to be less simple than hitherto suspected. Recent work by Morris and Williams (12) suggests that human blood contains appreciable quantities of the 17-desoxycorticosteroids, corticosterone and 11-dehydrocorticosterone, as well as the 17-hydroxycorticosteroids, cortisone and hydrocortisone.

Although the present investigation presumably covers a large proportion of the possible metabolites of the adrenocortical steroids in urine, there is the possibility that undetermined or unrecognized fractions could affect the validity of the interpretation of the present results.

It is believed, however, that in spite of the many objections the present studies are worthy of further investigation.

SUMMARY

1. The following fractions representing metabolites of the adrenocortical steroids in urine, have been measured: 17-ketosteroids, acid-stable formaldehydogenic substances and 17-ketogenic steroids.

2. The results suggest that there may be changes of quality as well as of quantity in adrenocortical secretion. Possible factors are the age of the subject and the nature of the stimulus.

3. This hypothesis has been discussed.

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17-KETOSTEROID AND PITUITARY FOLLICLE-STIMULATING HORMONE EXCRETION IN MYXEDEMA, BEFORE AND DURING TREATMENT WITH THYROXINE*

WILLIAM H. BEIERWALTES, M.D. AND RONALD C. BISHOP, M.D.

The Department of Internal Medicine, University of Michigan Hospital, Ann Arbor, Michigan

IN PATIENTS with myxedema, a decreased urinary excretion of 17-ketosteroids has been a uniform finding (1–4). Values under 3 mg. per day have been observed in women and under 7 mg. in men. The administration of desiccated thyroid to myxedematous patients has been reported to be ineffectual in restoring the excretion of steroids to normal, even when the clinical response to thyroid, including normalization of the basal metabolic rate and serum cholesterol level, was complete (1, 3, 5). Patients with hypothyroidism but without myxedema apparently have normal levels of urinary 17-ketosteroids (1). Several observers have found that in hyperthyroid persons the decrease in 17-ketosteroid excretion is not striking, the values being usually in the subnormal or low-normal range (1–4, 6). Satisfactory treatment of the hyperthyroidism is accompanied by a rise to normal of the 17-ketosteroids (1). No significant correlation has been noted between the clinical data, such as severity and duration of the thyrotoxic state, and the levels for urinary 17-ketosteroids (hereafter referred to as 17-KS).

Statland and Lerman (7) studied the excretion of pituitary follicle-stimulating hormone (FSH) in myxedematous patients. The values were generally within normal limits in men and in premenopausal women. Menopausal women tended to have somewhat lower levels of FSH than the expected high levels found in nonmyxedematous subjects. These values rose somewhat during thyroid therapy.

In the present investigation, 17-KS and FSH excretion were followed in myxedematous subjects who were given increasing doses of sodium-*l*-thyroxine,[1] to the point of simulated thyrotoxicosis. This was done in an attempt to raise the lowered 17-KS and FSH excretion to normal, and to ob-

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[1] The sodium-*l*-thyroxine, Elthrin, was generously supplied by Dr. John Mote, Dr. H. R. Knoch, and Mr. Edwin Boone, of Smith, Kline & French Laboratories.

serve any changes in these values during the development of simulated thyrotoxicosis. After the thyroxine medication was pushed to the point of toxicity, it was either decreased to nontoxic levels or stopped. This was done in order to observe the effect of relief of simulated thyrotoxicosis, or relapse into myxedema.

METHODS

Observations for this report were made on 20 consecutive myxedematous patients studied in the Medical Out-patient and In-patient Services of the University Hospital.

Fifteen of the patients were women ranging in age from 17 to 69 years, with an average of 47 years. Six of these women were in the period of active menstrual life, and 9 were menopausal. Five men were studied, their ages ranging from 32 to 52 years, with an average of 40 years. Eleven of the women and 2 of the men had primary spontaneous myxedema; the others had myxedema due to surgery or radioactive iodine. Pituitary myxedema was ruled out in each case by the history, physical examination, and laboratory tests, but most especially by the rise of 17-KS and/or FSH titers in response to the administration of thyroxine.

The method of Robbie and Gibson (8) was used in performing 17-KS determinations. The method for quantitative FSH determination was that of Klinefelter, Albright and Griswold (9). Each point in the charts to follow is usually the result of a single determination on one day. Table 1 summarizes the clinical and laboratory features of myxedema in each patient. Note that the main laboratory tests of thyroid function used were the basal metabolic rate, and the serum cholesterol and protein-bound iodine determinations. Table 1 shows the 17-KS and FSH excretion at the time of diagnosis. In addition, bone age, urinary estrogen excretion, eight-hour ACTH-test response, and glucose tolerance were determined in some patients. Sodium-*l*-thyroxine was then started by mouth, usually in a dosage of 0.05 mg. per day. The patient was generally seen at two-month to four-month intervals for about one year. The dosage of thyroxine was usually increased at each successive visit, first to 0.1 mg. per day, then to 0.25 mg. then to 0.5 mg. per day and, rarely, to 0.75 mg. or 1.0 mg. per day. Table 1 shows the maximum response to thyroxine obtained in each patient. Dosage of thyroxine was limited in some patients by the onset of angina pectoris, congestive failure, or lack of regularity in taking medications.

RESULTS

Urinary 17-ketosteroid excretion in women with myxedema

Figure 1 presents the 17-KS pretreatment values and maximum values during treatment in women. Generally, these data affirm previous reports that 17-KS excretion is lowered in patients suffering from myxedema. Three of 13 initial values, however, were above the lower range of normal, which for our laboratory is 5.1 mg. per twenty-four hours. Patient F. H. was menopausal and had an excretion of 15.1 mg. per twenty-four hours; she was receiving an inadequate dosage of thyroprotein at this time. Patient E. R., whose value was 6.1 mg. per twenty-four hours, was also menopausal, suffered from spontaneous myxedema, and was receiving an inade-

TABLE 1. SUMMARY OF THE CLINICAL AND LABORATORY FEATURES OF MYXEDEMA—17-KETOSTEROID (17-KS) AND PITUITARY FOLLICLE-STIMULATING HORMONE (FSH) VALUES IN EACH PATIENT BEFORE TREATMENT AND THE MAXIMUM RESPONSE DURING THE ADMINISTRATION OF THYROXINE

Patient	Age (yrs.)	Myxedema	Before treatment							Maximum response during treatment							Maximum dosage of sodium-*l*-thyroxine (mg./day)
			BMR (%)	Pulse	PBI (μg./100 ml.)	Cholesterol (mg./100 ml.)	17-KS (mg./24 hrs.)	FSH (mouse units)		BMR (%)	Pulse	PBI (μg./100 ml.)	Cholesterol (mg./100 ml.)	17-KS (mg./24 hrs.)	FSH (mouse units)		
Males																	
J.J.	38	Idiopathic	−32	54	4.7	197	13.4	+6	−48	−3	—	13.5	152	12.4	+6	−48	0.25
J.P.	32	Postop.	−32	50	2.0	310	11.4	−6	—	+3	64	9.6	128	20.2	+6	−12	0.5
R.N.	42	Postop.	−36	60	2.2	430	—	—	—	+18	80	14.8	177	10.6	+24	−48	0.1
A.S.	35	Idiopathic	−41	60	—	470	—	—	—	+2	80	—	193	16.9	—	—	0.75
G.S.	52	Postop.	−26	60	2.3	352	3.1	—	—	+1	80	5.6	220	7.0	—	—	0.1
	(Av. 40)																
Premenopausal Females																	
D.L.	17	Idiopathic	−36	52	2.9	579	3.5	−6.6	—	+14	78	24.5	140	4.2	−6	—	0.75
R.L.	33	Postop.	−27	82	—	635	5.5	+6.6	−53	+7	82	—	125	12.0	+12	−24	1.0
F.R.	32	Idiopathic	−24	50	3.9	269	1.0	+6	−48	+11	68	7.1	147	9.4	—	—	0.5
S.S.	18	Postop.	−49	56	—	472	3.3	—	—	−2	64	—	136	7.6	+6	−48	0.3
H.W.	42	Postop.	−15	60	2.5	308	4.6	+6	−48	+24	60	13.0	136	9.6	+12	−48	0.5
A.D.	18	Idiopathic	−25	68	1.1	339	2.5	−6.6	—	+52	120	11.8	129	6.3	+6	−24	0.5
	(Av. 29)																
Menopausal Females																	
H.B.	48	Idiopathic	−43	56	1.4	474	3.3	+6.6	−53	+6	80	3.1	169	2.1	—	—	0.25
F.H.	48	Idiopathic	−48	42	3.4	450	15.1	—	—	+9	68	14.0	133	6.6	—	—	0.5
J.H.	69	Idiopathic	−30	48	—	329	4.2	+96		−6	68	5.7	275	4.4	+96	−288	0.25
M.M.	58	Idiopathic	−33	48	—	412	7.2	+48		−10	60	5.7	266	6.5	+24	−48	0.25
E.R.	60	Idiopathic	−23	60	2.4	383	6.1	—	—	−2	76	—	—	—	—	—	0.1
C.T.	64	Postop.	−9	80	—	321	2.2	+192	−384	−6	—	—	257	8.2	+192	−384	0.25
V.W.	50	Idiopathic	−28	56	0.7	265	3.6	+6	−48	+17	68	15.9	135	7.1	+53	−106	0.5
M.B.	67	Idiopathic	−27	52	1.4	314	3.2	+48	−96	+17	88	7.8	137	4.9	+12	−24	0.1
G.R.	69	Idiopathic	−35	60	—	549	2.9	—	—	−3	96	—	254	5.7	—	—	0.25
	(Av. 58)																

quate dosage of thyroprotein. Patient R. L., whose daily 17-KS excretion was 5.5 mg., was premenopausal and had myxedema following radioiodine treatment for thyroid carcinoma. She had been off thyroid medication only one month at the time of the 17-KS determination.

Our data differ from previous reports in that 17-KS excretion by myxedematous women generally rose to normal (Fig. 1) when thyroxine dosage was increased sufficiently, even over periods as short as one and a half months. No definite difference in the response was observed between premenopausal and menopausal women with spontaneous or induced myxedema, regardless of age. Nine of 12 patients who had pretreatment and

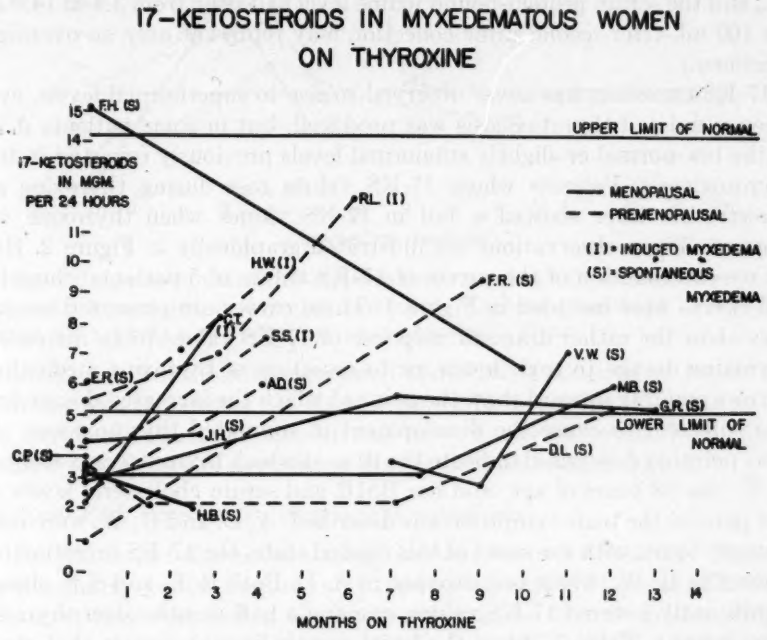

FIG. 1. 17-Ketosteroid excretion in women with myxedema—pretreatment values, and maximum values during treatment.

follow-up determinations of 17-KS showed a significant rise from the lowest to the highest 17-KS levels (Fig. 1), ranging from 2.8 to 8.4 mg., and averaging 4.7 mg. In Patients H. B. and D. L. there was an initial drop in 17-KS values during treatment. Although these patients showed objective evidence of some improvement of their myxedematous state, this improvement was slight. In D. L., when the dosage of thyroxine was later increased, there was a drop in the serum cholesterol level from 330 mg. to 140 mg. per 100 ml., and the 17-KS values rose to normal. This was also true of M. B. when the basal metabolic rate rose from −1 to +17 per cent and the

serum cholesterol level dropped from 167 mg. to 137 mg. per 100 ml. It is noteworthy that thyroxine had to be pushed to perhaps toxic levels in these patients before the 17-KS values rose to normal levels, even though they had previously been on apparently average doses of thyroxine for periods of seven months (D. L.) and eight months (M. B.). F. H. had an initial 17-KS excretion of 15.1 mg.—above the normal range. This value fell to 6.5 mg. after ten months of thyroxine therapy. At this time, her thyroxine dosage was 0.5 mg. per day, and her BMR had risen from −30 per cent with a basal pulse rate of 52, to +2 per cent with a basal pulse rate of 60. The serum cholesterol level had dropped from 335 mg. to 210 mg. per 100 ml., and the serum protein-bound iodine level had risen from 3.4 to 14.0 μg. per 100 ml. (Her second urine collection may represent only an overnight specimen.)

17-KS excretion was never observed to rise to supernormal levels, even when simulated thyrotoxicosis was produced, but in some patients it fell to the low-normal or slightly subnormal levels previously reported in true thyrotoxicosis. Patients whose 17-KS values rose during thyroxine administration later showed a fall in 17-KS values when thyroxine was stopped. These observations are illustrated graphically in Figure 2. Here we see continuation of the curves of 17-KS values of 5 patients whose initial curves were included in Figure 1. These curves are presented because they show the rather dramatic response of 17-KS values to an increase of thyroxine dosage to toxic levels, or to cessation of thyroxine medication. Arrows pointing upward show the point at which the increased dosage level was sufficient to cause the development of simulated thyrotoxicosis. Arrows pointing downward indicate the time at which thyroxine was stopped. C. T. was 68 years of age, and her BMR and serum cholesterol levels did not parallel the toxic symptoms she described. A. D. and H. W. were made severely toxic; with the onset of this clinical state, the 17-KS excretion rose further in H. W., whereas it dropped in A. D. Both R. L. and S. S. showed significantly lowered 17-KS values, one and a half months after thyroxine was stopped. Table 1 shows the basal metabolic rate, serum cholesterol, and serum protein-bound iodine responses in these patients during the same period of time.

Urinary 17-ketosteroid excretion in men with myxedema

Figure 3 shows 17-KS values in men. The data are scant but, generally speaking, they are not in line with previous reports that 17-KS excretion is lowered in myxedematous men. They are in the lower range of normal, however, and one man, G. S., did have subnormal values. Although J. P. showed a rise of 8.8 mg., no patient was observed to excrete 17-KS in supernormal amounts. J. P.'s 17-KS values continued to rise after the clinical picture of thyrotoxicosis was induced by administration of thyroxine. It

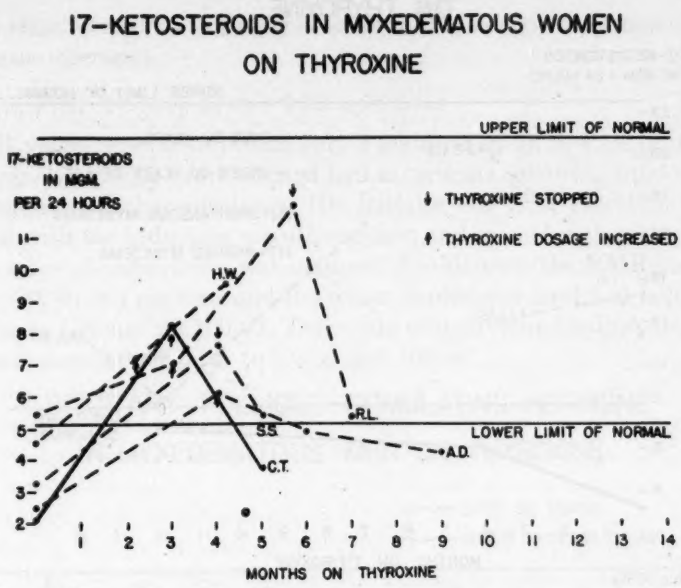

FIG. 2. Response of urinary 17-ketosteroids to increase of thyroxine dosage to toxic level, and to cessation of thyroxine medication. (Continued curves of 17-ketosteroid values of 5 patients whose initial curves are included in Figure 1.)

is interesting that the only subnormal 17-KS levels occurred in the one man who was past 50 years of age (G. S.). His 17-KS excretion (Fig. 3) rose during eleven months of treatment, but never to normal levels, even though he had no evidence of myxedema during the observation period.

Urinary FSH excretion in women with myxedema

The pituitary follicle-stimulating hormone titers are shown in Figure 4. In this chart, vertical lines connect the two titers of FSH tested. The point drawn at the lower end of the line represents the titer in mouse units at which the response was positive, and the point at the upper end of the line, the titer at which the response was negative. A horizontal line was then drawn arbitrarily half-way between these two data points to indicate the trend in FSH titer during the treatment of myxedema with thyroxine. These data simulate those of Statland and Lerman (7) in that most of the menopausal women (H. B., M. B., V. W. and M. M.) had FSH levels below those for women of this age (usually 96 to 480 mouse units per twenty-four hours). Also, all but 2 of the premenopausal women had FSH values within the normal range. Our data differ from those of Statland and Lerman (7) in that lowered menopausal FSH levels did not tend to rise during treatment with thyroxine and may actually have fallen in 4 patients. No signi-

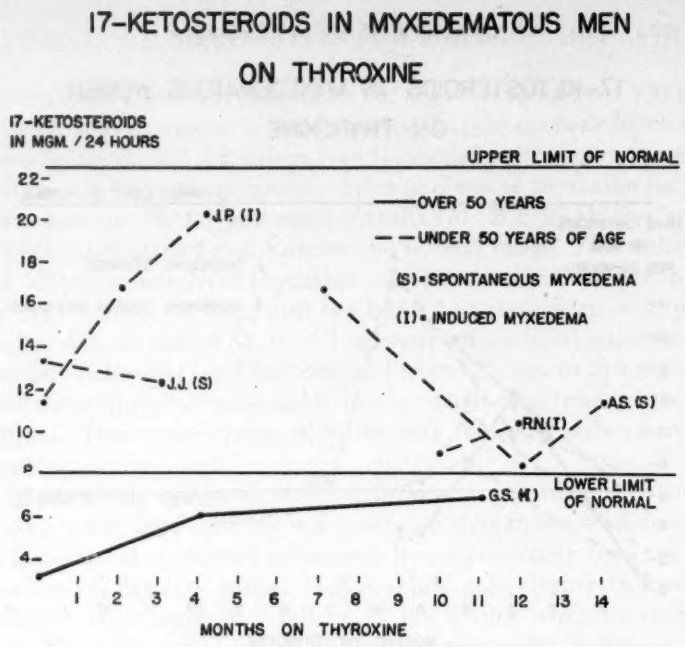

FIG. 3. 17-Ketosteroid excretion in men with myxedema.

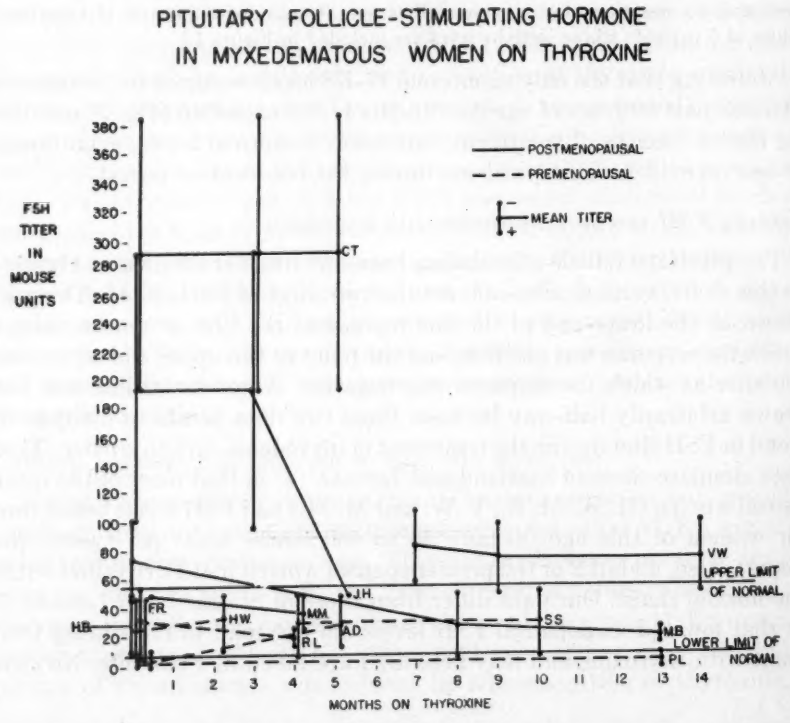

FIG. 4. Pituitary follicle-stimulating hormone titers in women with myxedema.

ficant trend is evident in the FSH values of premenopausal women during thyroxine therapy.

Urinary FSH excretion in men with myxedema

FSH values were obtained in only 3 males (Fig. 5). J. P. is of interest, however. He was 32 years old and had myxedema following total surgical and radioiodine thyroidectomy. His initially low FSH excretion rose to normal with the induction of euthyroidism and stayed within the normal range when thyrotoxicosis was induced. At this time the BMR had risen from −32 to +3 per cent and the serum cholesterol level had fallen from 310 mg. to 128 mg. per 100 ml. The serum protein-bound iodine concentration had risen from 2.0 μg. to 9.6 μg. per 100 ml.

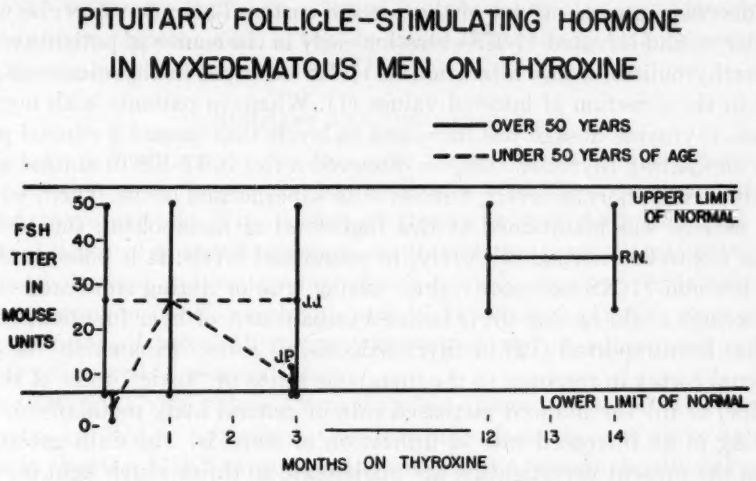

FIG. 5. Pituitary follicle-stimulating hormone titers in men with myxedema.

DISCUSSION

No substantiated explanation could be found in the literature for the observation that in myxedematous menopausal women the FSH excretion is lower than the expected high level in nonmyxedematous menopausal women. Theoretically, the formation or excretion of FSH by the pituitary could be decreased in myxedema as a result of hypometabolism of the pituitary cells, in common with other body cells. Moreover, the myxedema patient might have a longer biologic half-life of normal or decreased quantities of estrogen, and this increased effect of estrogen might prevent a rise in FSH levels to usual menopausal levels. In this connection, it is noteworthy that A. D., who had myxedema and subnormal FSH values initially, had urinary estrogen levels well within the normal range.

Some factor in the myxedematous state also seems to result in decreased excretion of 17-KS in women and possibly in elderly men. Theoretically, the cells of the adrenal cortex of the myxedematous person could be underactive due to the general decrease in metabolism associated with lack of thyroid hormone, or the adrenal cortex could be underactive due to the effects of the myxedematous state on the pituitary's production of ACTH. On the other hand, adrenocortical activity could be normal, but excretion of 17-KS decreased because of impaired liver function due to myxedema (10).

McGavack (11) originally suggested that patients with hyperthyroidism might first have a "compensated" stage during which they have adrenal hyperfunction, followed by a "decompensated" stage with an accompanying decrease in overactivity of the adrenal cortex. Later, however, he was unable to find elevated 17-KS excretion early in the course of patients with hyperthyroidism; if any alteration in 17-KS occurred in thyrotoxicosis, it was in the direction of lowered values (1). When, in patients with myxedema, thyroxine dosage was increased to levels that caused a clinical picture simulating thyrotoxicosis, we observed a rise in 17-KS to normal and rarely to high-normal levels, but never to supernormal levels. Then, while the patient was maintained at this high level of metabolism, the 17-KS often fell to low-normal or, rarely, to subnormal levels. It is possible that the lowered 17-KS excretion values during true or during simulated thyrotoxicosis could be due to: i) induced impairment of liver function, such as has been reported (12) in thyrotoxicosis, ii) some "exhaustion" of the adrenal cortex in response to the metabolic stress of "toxic" doses of thyroxine, or iii) the induced increased rate of general body metabolism, resulting in an increased rate of utilization of steroids. The data gathered from the present investigation are inadequate to throw much light on the mechanism which accounts for the rise or fall of FSH or 17-KS excretion in myxedema untreated, treated, or overtreated.

It seems worthwhile to point out, however, that 17-KS and FSH values may furnish a valuable clue to guide the clinician in his attempt to "normalize" the myxedema patient with desiccated thyroid or thyroxine. Usually the clinician raises the dose of desiccated thyroid until the patient says he feels well and the clinician thinks the patient looks well. If the dose of thyroid is raised very rapidly, the patient may have tachycardia, insomnia, and nervousness without losing the edematous appearance of the eyelids, carotinemia, and other clinical manifestations of myxedema; therefore, the development of tachycardia, insomnia, and nervousness is no guarantee that the symptoms of myxedema have been fully treated. In our series, occasionally treated myxedematous patients were observed to lose their subjective and objective manifestations of myxedema by the time of their

first return visit. At this time, lowered 17-KS values would be found and the dosage of thyroxine would be increased. On the next return visit, when 17-KS values had risen to normal, the patient would volunteer the information that even though he thought he was perfectly well at the time, of the previous visit, he now felt even better. It should be stressed, however, that under the conditions of our investigation, by the time myxedematous patients were made to look and feel well on thyroid therapy, more commonly than not they also showed normal 17-KS values.

Our data, though too few to warrant dogmatic statements, suggest that the person past 50 years of age with spontaneous myxedema is the patient who may require the longest period of time to raise urinary 17-KS levels to normal. If these values are indices of pituitary and adrenal cortex function, it would seem logical to attempt to "normalize" the myxedema patient in these respects, also, so that he might theoretically be better equipped to weather the usual stresses of life.

SUMMARY AND CONCLUSIONS

Twenty patients with myxedema were treated with increasing doses of sodium-*l*-thyroxine, frequently to the degree that they developed simulated thyrotoxicosis. The dosage of thyroxine was then decreased or stopped. Clinical observations were made and the basal metabolic rate, serum cholesterol and protein-bound iodine levels, pituitary follicle-stimulating hormone (FSH) excretion, and 17-ketosteroid (17-KS) excretion were determined before thyroxine was started, and at subsequent visits during the period of observation.

Our data on 17-KS values affirm reported observations that 17-KS excretion is lowered in myxedema. Our data differ from those of previous reports, in that the 17-KS excretion of myxedematous women usually did rise to normal when thyroxine dosage was increased sufficiently, even over periods as short as one and a half months. No definite difference in this response was noted between premenopausal and menopausal women with spontaneous or with induced myxedema, regardless of age. 17-KS levels did not rise to supernormal levels, even when simulated thyrotoxicosis was produced; but they dropped in some patients to the low-normal or slightly subnormal levels previously reported in true thyrotoxicosis. 17-KS levels of some women and one man, however, continued to rise, even when simulated thyrotoxicosis was produced. Generally, patients whose 17-KS levels did not rise to normal during treatment were receiving a lower daily dose of thyroxine than patients whose 17-KS levels rose to normal. Patients whose 17-KS levels rose during thyroxine administration had a subsequent drop in levels when thyroxine administration was stopped.

Our data on FSH values are scant, but agree with a report that FSH

excretion is within normal limits in males and in premenopausal women, but lower than the expected high levels in menopausal women. The main difference betweeen our data and those previously reported on FSH values in myxedema is that the lowered menopausal FSH levels did not tend to rise during thyroxine therapy and may actually have fallen in 4 patients. FSH levels did not rise to supernormal levels, even when the administration of thyroxine was increased to a dosage which produced a clinical picture simulating thyrotoxicosis. Only 1 man had a subnormal FSH excretion when myxedematous; this excretion rose to normal during treatment with thyroxine. Two premenopausal women had subnormal FSH levels before treatment; in 1 of these women, the level rose to normal during thyroxine administration. The mechanism of these changes in urinary FSH and 17-KS excretion in myxedematous subjects is not known.

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ANTITHYROID DRUGS PLUS ROENTGEN IRRADIATION IN THE TREATMENT OF HYPERTHYROIDISM

JOHANNES BÖE, M.D. AND ZETH GABRIELSEN, M.D.
The Medical Department, University Clinic of Bergen, Norway

ANTITHYROID drugs are now well established in the medical treatment of hyperthyroidism. Used in long-term treatment they give a fairly high proportion of apparent permanent remissions, by most authors calculated to be from 50 to 75 per cent. With the newer drugs of this type, complications are relatively few. In fact Doniach (1) treated 120 patients with a new drug of the methimazole group (Neo-Mercazole) without any complications.

The effect of roentgen irradiation in hyperthyroidism is widely disputed. Some authors are enthusiastic, whereas others find this treatment valueless. Pfahler (2) reports satisfactory results in 89 per cent of his patients; after only one month of such treatment he found some improvement, and after two months "very definite" improvement. Aagaard (3) records that 78 per cent of his patients had a remission. Rose (4), from his experience over a period of twenty years with 800 patients, states that satisfactory results were obtained in 85 per cent. Rasmussen (5) found that out of 13 patients treated for thyrotoxicosis by roentgen irradiation and iodine, and re-examined by him two years later, 12 were cured of the thyrotoxic factor. He regarded roentgen irradiation combined with iodine as a valuable treatment, although somewhat slow in effect.

On the other hand, McGavack (6) holds that good results can be expected only in one third of exophthalmic goiter patients treated with roentgen rays. Other authors have observed that, although improvement may take place in half the cases, total recovery is rare (7). Nielsen (8) says categorically that in his experience roentgen treatment of hyperthyroidism has not been of any value.

Complications following roentgen irradiation in hyperthyroidism seem to be rare. Rose and Wolferth (9), however, found 3 cases of mediastinocardiac reaction, and therefore advised against this treatment.

Rasmussen (5) suggested that roentgen treatment should be combined with administration of thiouracil. He regarded thiouracil therapy as purely symptomatic, whereas roentgen therapy was curative so far as the thyro-

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toxic factor of the disease was concerned. He felt that the use of x-rays in addition to thiouracil might shorten the period of medical treatment.

Although normal thyroid tissue is relatively resistant to irradiation, sensitivity seems to be greater when the gland is in a hyperactive state (2); therefore iodine treatment is not advisable before irradiation. When antithyroid drugs are used, however, the result is a reduction in the secretion of thyroid hormone and at the same time the rather paradoxical picture of an apparently highly hyperactive thyroid gland. Consequently, after treatment with antithyroid drugs one might expect a good result from subsequent roentgen therapy. Furthermore, as the effect of roentgen irradiation on the gland is rather slow, it might be advantageous to try to obtain at least a temporary remission by means of preliminary drug treatment.

In accord with this view, since 1948, all our cases of hyperthyroidism have been treated with antithyroid drugs plus roentgen irradiation. It has been possible to observe most of them for a sufficient length of time to enable us to evaluate the therapeutic results. The only report on such treatment that we have been able to find, is a preliminary one by Wahlberg (10) written in 1949, in which he states that promising results were obtained with 13 patients treated in this way.

CLINICAL MATERIAL AND METHOD OF STUDY

A few patients with large, unsightly goiters, particularly those in whom pressure on surrounding structure was present or expected to arise, were referred to the Surgical Department for operative treatment. Otherwise all patients with hyperthyroidism were given the combined treatment described. In all cases the diagnosis of hyperthyroidism was clearly established by clinical and laboratory data. Of a total of 140 patients, 115 were women and 25 were men. The sex and age distribution is shown in Table 1.

Table 2 gives a survey of the 140 subjects with regard to the eventual completion of treatment and control. Thirteen patients failed to return for control, after having been treated for a short time. Twenty-eight are still under treatment, and 19 of them have already been treated for one year or more. Observations on therapeutic response have been made in 99 patients. In 17 of these the combined treatment was given up for various reasons before it was completed, and other forms of treatment had to be given. The remaining 82 patients received long-term treatment, which was

TABLE 1. AGE AND SEX DISTRIBUTION IN 140 CASES OF HYPERTHYROIDISM

Age (yrs.)	15–19	20–29	30–39	40–49	50–59	60–69	70–79	*Total*
Females	1	13	26	31	28	13	3	*115*
Males	0	5	6	7	4	3	0	*25*
Total	*1*	*18*	*32*	*38*	*32*	*16*	*3*	*140*

TABLE 2. TREATMENT WITH ANTITHYROID DRUGS PLUS ROENTGEN IRRADIATION IN 140 PATIENTS

	No. of patients
Failed to return for control after initial treatment	13
Still under treatment	28
Treatment discontinued	17
Treatment completed	82
Total	*140*

discontinued when considered complete. In 73 of these cases the follow-up has been for more than six months. Data concerning toxicity have been collected from the entire series of 140 patients.

The initial classification of hyperthyroidism was based on the basal metabolic rate. As a rule each patient had several determinations of the metabolism made before treatment was started. The figures given in Table 3 are the results of the last recording in each case before treatment was started.

TABLE 3. DEGREE OF HYPERTHYROIDISM (BASED ON BASAL METABOLIC RATE)

		No. of patients	Per cent
Severe	(BMR above +60%)	30	21.6
Moderate	(BMR between +60 and +30%)	89	64.0
Mild	(BMR below +30%)	21	14.4
		140	*100.0*

According to the anamnesis, the patients had been ill for periods varying from a few months to more than five years.

Diffuse goiters were present in 91 per cent of patients. In 19 per cent they were classified as large, in 37 per cent as moderate and in 35 per cent as small. Nine per cent of patients had nodular goiters.

TABLE 4. DURATION OF SYMPTOMS BEFORE TREATMENT

	No. of patients	Per cent
Less than six months	36	25.7
Six to twelve months	55	39.3
One to five years	35	25.0
More than five years	14	10.0
	140	*100.0*

Twenty-four patients had received previous treatment for hyperthyroidism. In 8 of them the disease had relapsed after previous subtotal thyroidectomy. Two of these patients had also been treated with methylthiouracil, another had been operated on twice and in the interval had received iodine, roentgen treatment and methylthiouracil. In the iodine-treated group, roentgen irradiation had been added in 4 cases.

TABLE 5. PREVIOUS TREATMENT RECEIVED BY 24 PATIENTS

	No. of patients
Subtotal thyroidectomy	8
Iodine treatment	14
Roentgen treatment only	1
Roentgen treatment and methylthiouracil	1
	24

TREATMENT

During the first period of this investigation, in addition to roentgen irradiation, methylthiouracil was used; later this was changed to propylthiouracil, and finally Tapazole (methimazole). In some cases treatment was changed from one drug to another because of sensitivity, or because of presumed lower toxicity. Table 6 shows the number of patients who received the different drugs and combinations of drugs.

a) *Dosage of antithyroid drugs*

The initial daily dosage of methylthiouracil was 600 milligrams, of propylthiouracil 400 milligrams, and of Tapazole 15 milligrams, divided into three doses and taken at intervals of approximately eight hours. This dosage was generally continued for about three weeks; then it was gradu-

TABLE 6. ANTITHYROID DRUGS USED IN COMBINATION WITH ROENTGEN IRRADIATION

		No. of patients
Methylthiouracil		60
Propylthiouracil		23
Tapazole		28
More than one drug:		
Methylthiouracil and propylthiouracil	17	
Methylthiouracil and Tapazole	7	
Propylthiouracil and Tapazole	2	
Methylthiouracil, propylthiouracil and Tapazole	3	29
Total		*140*

TABLE 7. DURATION OF TREATMENT IN 67 CASES REGARDED AS CURED

	No. of patients	Per cent
Less than 6 months	4	6.0
Six to 12 months	24	35.8
Over 12, to 24 months	25	37.3
More than 24 months	14	20.9
Range: 3 to 60 months.		
Average: 14.4 months.		

ally reduced—the reduction being based more on the clinical appraisal of the patient than on determination of the basal metabolic rate. When after a few months (three, on the average) the disease was evidently under control, the patient was given a maintenance dose. This varied in different cases, but as a rule was: methylthiouracil 100–200 milligrams daily, propylthiouracil 50–100 milligrams daily, or Tapazole 5 milligrams daily.

The duration of treatment varied widely from three months to five years. For the 67 patients regarded as apparently cured, the average duration of treatment was 14.4 months.

Discontinuance of treatment was not considered unless the patient had been on the maintenance dose for at least two months. Usually treatment was terminated only after considerably longer periods.

b) *Roentgen treatment*

When clinical improvement became apparent as a result of antithyroid drug therapy (usually within four weeks) and when, as a rule, the basal metabolic rate had fallen below +30 per cent, roentgen irradiation was started.

Roentgen therapy was given under the direction of Professor S. Bakke, of the Department of Radiology. The technique was as follows: 175 K.V., 4 M.A., filtration 2 mm. Cu, distance 30 cm., 3 fields. From 200 to 300 r were given each day for nine consecutive days, the total dose being 1800 to 2700 r. Within these limits the dosage varied according to the size of the goiter. Apart from a mild local erythema and a slight and transient hoarseness, no untoward reactions were observed.

After discharge from the Department, the patients were subjected to ambulatory control. As a rule they returned at intervals ranging from four to eight weeks, until an euthyroid status was achieved. Later on, mainly because of difficult geographic conditions, the control was more irregular and the intervals between visits became longer—from two to six months. The average period of control after discontinuance of treatment for the group considered permanently cured was 15.9 months.

RESULTS

Complications

For the total group (140 individuals), untoward effects were as shown in Table 8.

TABLE 8. UNTOWARD EFFECTS OF ANTITHYROID DRUGS IN 140 PATIENTS

Drug	Exanthema or itching			Nausea, vomiting			Leukopenia			Fever			Agranulocytosis			*Total*			
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	*A*	*B*	*C*	
Methylthiouracil	1	3	1	2			1	1		2	5			3	4	*6*	*12*	*5*	*23*
Propylthiouracil	1			1			1		1			1				*3*		*2*	*5*
Tapazole		1					1					1		1		*1*	*2*	*1*	*4*
Total	*2*	*4*	*1*	*3*			*3*	*1*	*1*	*2*	*5*	*2*		*4*	*4*	*10*	*14*	*8*	*32*

A = Treatment temporarily discontinued; the same drug later resumed, and tolerated.
B = Treatment discontinued; later resumed with another drug without untoward effects.
C = Complete cessation of antithyroid drug therapy. Started on iodine, surgery or I^{131}.

The frequency of untoward effects of the different drugs was:

Methylthiouracil	26.4 per cent
Propylthiouracil	11.1 per cent
Tapazole	10.0 per cent

In 10 cases treatment was resumed without the reappearance of allergic reactions. In 14 cases therapy could be continued with another drug, but in 8 cases the treatment had to be stopped because of complications.

In 1 patient treated with Tapazole, agranulocytosis developed after four weeks' treatment. This patient recovered and was later treated with propylthiouracil without untoward effects. Agranulocytosis following Tapazole therapy is considered very uncommon (1).

Effectiveness of treatment

The rate and degree of response to the antithyroid drugs varied widely from one individual to another, but no case was refractory to treatment by these drugs. In 17 cases the treatment by antithyroid drugs was discontinued for various reasons, mostly because of untoward effects (Table 8), impatience and lack of cooperation. These cases were then referred for surgical treatment or iodine therapy, or were given I^{131}.

Table 9 gives the figures for the 99 patients whose treatment was discontinued either because it was considered adequate or because untoward effects made another form of treatment imperative. Sixty-seven of the patients had apparently permanent remission. All of them were kept under observation for more than six months after discontinuance of treatment. Four patients treated and observed in this manner were improved but could not be regarded as cured. Eleven others were apparently cured, but

TABLE 9. RESULTS OF COMBINED TREATMENT IN 99 PATIENTS WITH HYPERTHYROIDISM

	No. of patients
Cured (control period more than six months)	67
Improved	4
Probably cured (observation period less than six months)	11
Failures (untoward effects, impatience, lack of co-operation, recurrences)	17
	99

they have not yet been under observation for the full six months after cessation of treatment. In our material relapses occurred 45 times in 35 patients.

More than 50 per cent of the relapses occurred within four months after discontinuance of drug therapy (Table 10). Of the 8 relapses which occurred more than six months after discontinuing the drug therapy, 5 were later successfully treated with a new series of antithyroid drugs plus roentgen irradiation.

DISCUSSION

Reports and figures concerning the value of both drug and x-ray therapy in hyperthyroidism vary so widely that it is difficult to estimate if a combination of the two forms of treatment, as used in this series, is better than either of them used separately.

One of the reasons for the large differences shown in the results is that, although laboratory methods can be used up to a point, the ultimate decision depends upon clinical appraisal and therefore involves a certain amount of subjectivity. It is also difficult to differentiate between relapses, "false recurrences" (11), and "true recurrences" by using a six-month limit. All degrees of transition exist, as shown by representative reports in

TABLE 10. RELAPSES OBSERVED

Period of treatment	Relapses observed after cessation of treatment			
	Less than 4 months	4–6 months	More than 6 months	*Total*
Less than 6 months	7	4	1	*12*
Six to 12 months	8	3	4	*15*
Over 12, to 24 months	8	6	3	*17*
More than 24 months	0	1	0	*1*
Totals	*23*	*14*	*8*	*45*

this field (10–15). Since recurrences may occur even after years of treatment, and after years of complete freedom from symptoms, there will always be some uncertainty about figures given for complete recovery.

With these reservations, the results shown in our series seem to compare with the best results reported for long-term treatment with antithyroid drugs (11, 13, 14, 15) and for treatment with roentgen irradiation.

A careful appraisal of our results indicates that out of 100 patients subjected to this combined treatment, 75 would show a permanent remission 5 would show improvement, and in 20 the treatment would have to be given up and other forms of therapy substituted.

The addition of roentgen irradiation to antithyroid drug therapy does not seem to shorten appreciably the period of treatment necessary to obtain a permanent remission. The average period of treatment in our series was fifteen months. Using methylthiouracil, Iversen (11) reported 11.8 months as the average duration of treatment; and Wing *et al.* (15), using propylthiouracil, found the average duration of therapy to be 24.7 months.

Long-term treatment of hyperthyroidism with antithyroid drugs is far from being ideal, and our experiences indicate that the addition of roentgen therapy does not obviously improve the results. However, as roentgen irradiation of this type is practically harmless, easy to administer, and may possibly help to bring about permanent remissions, it may be used as an adjunct to conservative treatment with antithyroid drugs.

SUMMARY

In the Medical Department of the University Hospital of Bergen, 140 patients with hyperthyroidism have been treated with antithyroid drugs—methylthiouracil, propylthiouracil or methimazole (Tapazole)—plus roentgen irradiation.

Methylthiouracil was given to 60 patients, propylthiouracil to 23, and Tapazole to 28. Twenty-nine patients were treated in succession with two or three of the drugs. Seven cases of agranulocytosis occurred with methylthiouracil, and 1 with Tapazole; none was fatal.

Of 99 patients whose treatment was concluded, 67 showed permanent remissions. They were all observed for at least six months after terminating therapy, and the average period of observation was 15.9 months. Eleven other patients showed apparently permanent remissions, but they have not been observed for six months after discontinuance of treatment. Four patients were improved. In 17 cases the treatment was given up for various reasons (untoward reactions, recurrences, impatience, lack of co-operation), and other forms of therapy substituted.

The average duration of treatment for the patients with apparently permanent remissions was 14.4 months.

Roentgen irradiation in addition to long-term treatment with antithyroid drugs does not seem to offer a distinct advantage over antithyroid drug therapy alone. It does not seem to shorten the period of treatment needed to produce permanent remissions. It is, however, possible that it increases the number of permanent remissions and, as it has practically no disadvantages, it may serve as additional treatment when conservative therapy with antithyroid drugs is used.

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A FOUR-YEAR STUDY OF THE TREATMENT OF HYPERTHYROIDISM WITH METHIMAZOLE*

JACQUELINE CHEVALLEY, M.D., THOMAS H. McGAVACK, M.D., SAMUEL KENIGSBERG, M.D. AND SIDNEY PEARSON, PH.D.

The Department of Medicine, New York Medical College and the Metropolitan Hospital Research Unit, Welfare Island, New York 17, N. Y.

AS a result of Stanley and Astwood's (1) observations on the inhibitory effect of 1-methyl-2-mercaptoimidazole (methimazole) on the uptake of radioiodine by the human thyroid, early in 1949 we began to test the value of this and several closely allied imidazoles in patients with thyrotoxicosis. Using technics developed by Astwood and his associates (1–3), we simultaneously studied the relative intensities of the inhibitory effects of these compounds upon the uptake of radioiodine by the human thyroid (4). As a result of these early studies, it was decided to test the long-range usefulness of methimazole in the management of thyrotoxicosis. Since the earlier reports (1, 4–9), several articles have appeared regarding the effect of this drug in the treatment of hyperthyroidism (10–25). Although these represent studies with approximately 700 patients, most of the reports refer to short-term observations. Moreover, there is considerable divergence of opinion regarding the effectiveness and the toxicity of the compound. Conflicting claims that 1) it is too toxic for other than preoperative use (19), and 2) no untoward reactions have been seen (4, 5, 10), have appeared almost simultaneously. A summary of a long-term study seems, therefore, all the more timely.

METHODS AND PROCEDURES

In 184 patients, the diagnosis of thyrotoxicosis was established by methods previously described (26–30). Of these 184 patients, 141 or 76.6 per cent were women, 40 or 21.6 per cent were men, and in 3 the sex was not recorded. They varied in age from 15 to 83 years; the average age of the women was 41.6 years and of the men, 40.3 years.

Forty-three subjects were hospitalized at the outset of treatment with methimazole[1]

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for periods of time varying from two weeks to several months, not because of greater severity of the disease, but with a view to making more continuous and thorough investigation of the effects of the antithyroid compound.

In the first 60 patients, a dose of from 5 to 20 milligrams of methimazole was given daily; most patients were started at the 15-milligram level. Seventeen patients were thus prepared for surgery. Of the remaining 43, 26 were eventually brought under control with the amounts mentioned, and in 17 this was increased rapidly, usually at the end of the first or second week of treatment. In all additional subjects, initial doses varied from 30 to 40 milligrams. Rarely was it necessary to increase the amount to 60 milligrams daily to control the thyrotoxic phenomena.

"Maintenance" doses of methimazole varied from 2.5 to 30 milligrams; most of the subjects required about 20 milligrams daily for a steady continuous effect. Iodine in the form of Lugol's solution, not more than 1 minim daily, was added to the regimen when the patient became euthyroid or virtually so; simultaneously a decrease in the dosage of methimazole was gradually effected. The iodine was continued for not less than three months after treatment with the antithyroid compound was discontinued.

Initially, 37 of the 184 patients were prepared for subtotal thyroidectomy; the remainder were treated with a view to appraisal of long-term medical management with methimazole.

In order to ascertain the influence of age on the therapy of hyperthyroidism with an antithyroid compound, these cases were arbitrarily divided into three groups: (a) those between 15 and 34 years of age, of whom 35 were women and 11 were men; (b) those between 35 and 54 years of age, of whom 64 were women and 20 were men; and (c) those from 55 to 83 years of age, of whom 38 were women and 7 were men.

A diffuse hyperplasia of the thyroid existed in 98 of the 184 patients, of whom 75 were women and 23 men. A grossly nodular gland could be palpated in 62 instances, of which 51 were in women and 11 in men. In 6 patients, 4 men and 2 women, no thyroid tissue could be demonstrated in the neck but was present substernally as shown by x-ray examination and radioiodine uptake. No thyroid tissue was demonstrable in the neck on physical examination in 11 additional patients, of whom 9 were women and 2 were men. The thyroid appeared to be normal in 3 women. The type of gland was not recorded in 4 patients. Twenty patients, 3 men and 17 women, previously had undergone thyroidectomy.

RESULTS

I. *Thyrotoxic patients treated preoperatively only*

Of 37 patients initially prepared for surgery, 35 had a nodular goiter, 2 a hyperplastic goiter. Seventeen of these patients were prepared for operation with initial daily doses of methimazole which did not exceed 20 milligrams; most of them received 15 milligrams daily. Of these, 11 required from five to thirteen weeks to be prepared for surgery, whereas 6 needed less than five weeks. This is in sharp contrast to the remaining 20 patients prepared for operation with doses of methimazole varying from 30 to 60 milligrams daily, in no one of whom was more than five weeks required for

completion of surgical preparation. Our present routine is to start the patient on 30 milligrams of drug daily in 3 equally divided doses, and to increase the dose at the end of the first week if there is no change in the clinical condition. Complete control of the thyrotoxic state was attained as readily in those patients with nodular goiter as in those with a "hyperplastic" gland. The speed with which control was achieved bore no mathematical relation to the initial severity of the condition as gauged by the basal metabolic rate or other diagnostic criteria. Indeed, extremely toxic patients were often the first to note improvement and in many instances returned to a euthyroid state more promptly than their less toxic fellows.

II. *Thyrotoxic patients in whom long-term therapy with methimazole was attempted*

(a) Time required to effect an initial improvement in the clinical status of the patient

The number of days of treatment which passed before a definite improvement in the patient's condition could be noted were recorded in 150 of the 184 patients, or in 81.4 per cent (Table 1). Usually the patient volunteered information about any improvement he had experienced, and the most common first remark was that "the inner tension has been relieved." Until this was accompanied by amelioration of some of the other presenting signs or symptoms, such as tremors, hyper-reactivity, sweating, tachycardia, disturbances of appetite and bowel, or altered sleep, the patient was not considered improved. Reliable decreases in the basal metabolic rate and increases in the serum total cholesterol level usually occurred after other evidences of improvement were clearly marked, which indicates that these tests are not too sensitive and are also subject to certain technical errors.

There was a direct connection between the size of the dose and the speed

TABLE 1. SPEED OF ACTION OF METHIMAZOLE IN 184 CASES OF HYPERTHYROIDISM

Age group	Total No. of patients	Time required for onset of definite improvement (wks.)					Minimum time required to establish control (wks.)									
		Total No. of records	1	2	3–4	5–8	Total No. of records	Disease not controlled	2–3	4	5	6	7–8	9–10	11–13	14–26
15–34	46[12]*	40[10]	26	14[10]	0	0	38[10]	1	12	14[2]	3[1]	5[4]	2[2]	1[1]	0	0
35–54	84[16]	69[9]	29	31	6[6]	3[3]	63[9]	2	17	23	17[5]	0	1[1]	0	2[2]	1[1]
55–64	45[12]	38[10]	20[1]	16[7]	2[2]	0	33[10]	0	9	13[1]	2[1]	3[2]	1[1]	3[3]	2[2]	0
Age not recorded	9[3]	3[3]	2[2]	1[1]	0	0	3[3]	0	2[2]		1[1]	0	0	0	0	0
Totals	*184[43]*	*150[32]*	*77[3]*	*62[18]*	*8[8]*	*3[3]*	*137[32]*	*3*	*40[9]*		*51[4]*	*22[7]*	*8[6]*	*4[4]*	*4[4]*	*1[1]*

* The numbers in brackets refer to the early patients who received maximum doses of 20 milligrams of methimazole daily.

with which improvement began. Of the 60 patients who received initially doses of 20 milligrams or less, 17 received increased doses before any appreciable clinical alteration could be detected. The day on which the patient first observed a change for the better in his condition was recorded in 32 of the remaining 43 (Table 1). The first definite signs of improvement were observed during the first week in 3 patients, during the second week in 18, during the third or fourth week in 8, and somewhere between the fifth and eighth weeks in 3. This is in sharp contrast to the speed with which amelioration was noted during administration of larger doses of drug in the 118 patients for whom information on this point is available. Seventy-four of these were definitely better during the first week of therapy and the remaining 44 during the second week (Table 1).

Of the 150 patients for whom the date of "first improvement" was recorded, 77 showed both subjective and objective evidences of "feeling better" during the first week, an additional 62 during the second week, and the remaining 11 within the six weeks which followed. Sometimes the loss of thyrotoxic manifestations was noted as early as the end of the first day, but usually little change was observed until the third or fourth day. From this time forward, the patient, if receiving a sufficiently large dose, progressed rapidly. Methimazole is by far the most rapidly acting antithyroid compound with which we have had experience (23). 1-Methyl-2-carbethoxythio-imidazole may prove equally effective. Incidentally, we could see no difference in the time required to control the toxicity associated with a nodular goiter and that present in a diffusely hyperplastic one.

(b) Time required to induce a euthyroid state

We considered a patient euthyroid as soon as clinical findings of hyperthyroidism had disappeared and the basal metabolic rate and serum cholesterol level were both within normal limits. On this basis, observations were made in 137 of the 184 patients (Table 1), including 32 of the 60 patients who were initially given doses of 20 milligrams or less. Of the 137 patients, 3 were never satisfactorily controlled with the antithyroid compound, 40 became euthyroid between the twelfth and twenty-first days of treatment, 51 betweeen the twenty-first and twenty-eighth days, 22 during the fifth week, 8 during the sixth week, and the remaining 13 between the seventh and twenty-sixth weeks (Table 1). Thus, full control of the thyrotoxic state was achieved within five weeks in 113 of 137 patients, or 82.4 per cent. If we exclude those patients who received a rather small dose of the drug, then 100 of the 105 remaining patients, or 95.2 per cent, were completely controlled by methimazole within five weeks of initiating therapy, and approximately 40 per cent were euthyroid by the end of the third week of treatment. The 3 patients in whom the drug was ineffectual in controlling

the thyrotoxicosis were the 3 who showed a granulocytopenic response while taking methimazole.

(c) Comparison of some clinical findings in the thyrotoxic and euthyroid states

In 151 of 180 patients, or 83.8 per cent, the *basal metabolic rate* was above +20 per cent when therapy was begun and the highest recorded value was +94 per cent. Of the 29 patients with a metabolic rate below +20 per cent at the time administration of methimazole was started, 21 had been previously controlled by another antithyroid compound and had been transferred to methimazole therapy for maintenance and further study. The remaining 8 were thyrotoxic, as gauged by all of the criteria available; in 1 of these it was necessary to depress the basal metabolic rate to −15 per cent before a euthyroid state was attained. This patient is still receiving antithyroid medication (twenty-eight months after beginning therapy) and is well, although her basal metabolic rate remains between −15 and −5 per cent.

A tendency to *gain weight* as the manifestations of hyperthyroidism disappear, is noted in most hyperthyroid subjects; and the reversal of the steady weight loss commonly seen in these individuals is among the earliest signs of improvement. Of 168 patients in whom changes were followed serially, 138 or 82 per cent gained weight, 17 lost weight and 13 showed no change.

Prior to treatment with methimazole, the *pulse rate* was below 86 beats per minute in those 20 subjects who had been previously taking maintenance doses of some other antithyroid compound. After treatment, a pulse above 86 was found in 58 of 181 patients in whom the pulse was recorded. It is important to note that control of the hyperthyroid state was considered satisfactory in 42 of these 58 patients. In the other 16, the patient had not been treated sufficiently long or intensively for complete control of symptoms.

A high systolic and a low diastolic *blood pressure* is the rule in uncomplicated Graves' disease and very common in all forms of hyperthyroidism. The reversion of the pulse pressure to normal is a useful confirmatory sign of improvement. In 114 of 169 patients the pulse pressure was above 60 before treatment, whereas in 119 of 155 patients the pulse pressure was below 60 after adequate therapy with methimazole.

We have previously called attention to the behavior of the *serum cholesterol level* in connection with therapy with antithyroid compounds other than methimazole (26, 29, 30). In the present series of cases, values for serum cholesterol, even though initially within normal range rose during

treatment in 99 of the 115 subjects upon whom serial determinations were made.

(d) Alterations in the size of the thyroid during treatment

In the records of 90 patients, mention was made of the size of the thyroid gland during treatment, In 12 cases (7 controlled) there was an increase; in 45 (all well controlled), a decrease; and in 33 (27 well controlled), no change. In 20 of these subjects it was believed that an early slight increase in the size of the gland was followed by a definite decrease, as full control of the thyrotoxic state was attained.

(e) Final disposition of cases

The final disposition of 133 of the 184 cases has been tabulated in relation to the duration of treatment and to the age of the patients (Table 2).

TABLE 2. FINAL DISPOSITION OF 133 CASES OF HYPERTHYROIDISM TREATED WITH METHIMAZOLE

Duration of treatment (wks.)	Total No. of patients	Disposition		Receiving Methimazole		Relapses*		Recurrences†		"Cures"‡		Operations		No. of patients receiving I^{131}
		Known	Unknown	Yes	No	Number	Time until relapse (wks.)	Number	Time until recurrence (wks.)	Number	Length of follow-up (mos.)	Number	Length of preparation (days)	
2– 4	47	35	12	7	28	3	4.3	0	—	1	7	26	19.1	1
5– 13	49	30	19	14	16	6	3.8	2	23.5	0	—	11	39.3	3
14– 26	21	11	10	3	8	4	6.0	2	39.0	3	17.5	3	?	0
27– 52	29	22	7	5	17	8	3.8	2	5.0	12	11.9	2	?	1
52–104	23	20	3	7	13	2	7.0	1	60.0	10	16.0	1	?	1
104–156	12	12	0	4	8	3	7.3	1	15.0	6	15.0	1	?	0
More than 156	3	3	0	1	2	3	5.3	0	53.0	2	16.5	0	?	0
Totals	*184*	*133*	*51*	*41*	*92*	*29*	*4.9*	*8*	*32.2*	*34*	*14.4*	*44*	*?*	*6*
According to age groups														
15–34	46	37	9	12	25	9	4.7	2	30.0	13	15.0	9	20.0	1
35–54	84	62	22	17	45	14	4.1	5	28.3	14	16.2	24	47.5?	2
55–85	45	33	12	12	21	6	6.0	1	60.0	7	11.2	10	17.7	3
Age not recorded	9	1	8	0	1	0	—	0	—	0	—	1	35.0	0
Totals	*184*	*133*	*51*	*41*	*92*	*29*	*4.9*	*8*	*32.2*	*34*	*14.4*	*44*	*?*	*6*

* Relapse = Exacerbation or recrudescence of hyperthyroid state within four months after the cessation of therapy.
† Recurrence = Return of hyperthyroid state later than four months after cessation of therapy.
‡ "Cure" = Freedom from all evidence of hyperthyroidism for more than four months after cessation of therapy.

Of the 133 patients followed, 41 are still receiving methimazole; 92 are not. To determine the long-range effects of methimazole it was necessary first to eliminate the 37 patients in whom subtotal thyroidectomy was initially planned. Of the 96 remaining patients successfully followed, 41 are still receiving methimazole. In other words, 55 patients completed a course or courses of therapy with the drug.

No patient was listed as cured, in whom a recurrence was observed within the period of follow-up. With this restriction, 34 patients recovered completely *i. e.*, were "cured." In addition to these 34, 8 patients recovered completely, only again to experience the onset of hyperthyroidism when subjected to precipitating factors similar to, or identical with those which heralded the first attack. In the strictest interpretation of our definitions, such cases might be classified among the cures; however, they were not included in any of the percentages in this study. From Table 2, it will be noted that of 96 cases finally available for long-range therapy with methimazole, 41 are still under active treatment. Of the 55 patients in whom treatment was completed, 34 (61.8 per cent) remain symptom-free after an average of fifteen months following the cessation of therapy. This figure for recovery may be unduly weighted, in view of the fact that no satisfactory follow-up was obtained in 51 patients in whom methimazole therapy was started. If we assume that all of these were failures, then the percentage of recovery may be expressed as 34 divided by 55 plus 51 and multiplied by 100, or 32.1 per cent. The actual results which can usually be obtained by therapy with methimazole probably lie somewhere between these two percentages.

Table 2 shows that very few cures occur in patients who are treated for less than six months. This emphasizes the point that no medical regimen should be initiated unless both patient and physician understand that from six to eighteen months will be necessary for the completion of a single course of therapy.

(f) Effects of methimazole as influenced by age

At similar levels of dosage, the middle-aged group was the one in which it took longer (statistically) for methimazole to effect an initial response and to bring about complete control of thyrotoxicosis (Table 1). From a review of the clinical data, one gains the impression that the young group behaved in a more labile fashion than the two older groups, *e.g.*, the serum cholesterol level and the body weight rose more rapidly in a higher percentage of cases, and the pulse rate and pulse pressure returned to normal values more readily and in a greater number of subjects. These changes in the pulse rate and pulse pressure are highly significant.

The enlarged gland of hyperthyroidism decreased in size in 15 of 23 patients in age-group I (15–34 years old), in 16 of 37 in age-group II (35-54 years old), and in 10 of 24 in age-group III (55–83 years old). This difference between group I and the other two groups is at least in part related to the fact that diffusely hyperplastic glands are more common in the earlier decades of life.

After removal of the 37 cases in which methimazole was employed only

preoperatively, the percentage of "cures" in each of the three groups (from youngest to oldest) was 40.6, 36.0 and 28.0, respectively (Table 2). In view of the small number of cases, this represents no real difference between Groups I and II, but is statistically significant when Group I is compared with Group III.

(g) Toxic reactions

In 8 patients, or 4.3 per cent, untoward reactions to methimazole developed. Four patients had pruritis and localized rashes (1 of these also had edema); 3 had granulocytopenia; and 1 had a generalized urticarial febrile reaction. In 3 of these 8 patients—the one with an urticarial febrile episode and two of those who had granulocytopenia—the reactions could be classified as severe. The others were moderate to mild in degree and did not preclude the use of other antithyroid compounds. The most severe reactions occurred in patients receiving 40 milligrams or more of methimazole daily. We have always looked upon the urticarial febrile reactions as instances of hypersensitivity, so we are not inclined to accept these as quantitatively related to the dosage used, whereas the granulocytopenias seem to represent a direct toxic effect of the drug on the bone marrow. The cases with granulocytopenia (3 in number, or 1.6 per cent) all started with a slight fever, sore throat, anorexia and generalized malaise. In no instance did the condition progress to agranulocytosis. The symptoms at onset differed little, unless perhaps quantitatively, from those seen in the agranulocytosis associated with the administration of other antithyroid compounds. One gains the impression that methimazole is less toxic to bone marrow than are some of the other antithyroid compounds in equivalent therapeutic doses, and less liable to cause complete suppression of circulating granulocytes.

Treatment of granulocytopenia consists in forcing fluids and giving 400,000 units of penicillin every four to eight hours, depending upon the severity of the case. In our hands the highly recommended quasi-specific medications, such as pyridoxine, pentnucleotide®, liver, or folic acid, have not seemed to make the slightest difference in the speed or intensity of the recuperation of the bone marrow. At no time, did we consider any of the patients with a reaction to methimazole critically ill.

DISCUSSION

In order to replace previously employed medications, an antithyroid compound must possess some decided advantages. Among the factors to be considered in such an evaluation are (a) speed of action, (b) facility of maintenance, (c) net influence on the thyroid gland, and (d) incidence of toxicity. What can be said of methimazole in regard to these points?

(a) *Speed of action.* In patients with hyperthyroidism, both subjective and objective improvement occur more rapidly with methimazole than with any of the thiouracils (7, 12, 19, 21, 24, 31), including iodinated thiouracil (25). The time required to effect a remission also seems to be much, shorter than with other commonly employed preparations (7, 12, 17, 19, 21, 24, 25, 31). Although such action is highly desirable under all circumstances, it is of greatest advantage in preoperative care, in aged subjects and in those with cardiac failure. Furthermore, it is useful in rapidly restoring control of the disease in those cases in which there has been a relapse during treatment. This rapidity of action is perhaps the greatest single recommendation for the use of methimazole. A new imidazole derivative, 1-methyl-2-carbethoxythio-imidazole, shows promise of maintaining such rapidity of action, with a lower incidence of side effects than has been obtained in connection with the administration of methimazole (32, 33).

There seems to have been a general belief that the toxicity of a nodular goiter is not as readily influenced by antithyroid compounds as that of the diffusely hyperplastic gland (11, 12, 17, 25)—a belief which has been made the basis by at least one group of workers for determining the initial dosage of antithyroid drug (11). Hallman and Bondy (12) found this differential in dosage necessary when using propylthiouracil, but they emphasized that methimazole is just as effective in controlling the toxicity of nodular goiter as of diffusely hyperplastic goiter. This would be in accord with the experience presented here. Such observations afford further proof that antithyroid compounds vary in their modes of action, one from the other (19, 34).

(b) *Facility of maintenance.* With the smaller doses earlier employed (6, 31) some difficulties were encountered in maintaining good control of hyperthyroidism by the use of methimazole; but with adequate dosage, the clinical condition can be as well maintained as with methylthiouracil and better than with the other commonly employed compounds (10, 24). Maintenance doses recommended at present are in accord with those of Kendrick, Balls and Rose, who found that remissions could be "maintained without difficulty" by employing daily doses varying from 10 to 20 milligrams (17).

(c) *Net influence upon the thyroid gland.* In the cases recorded here, a decrease in size of the thyroid was noted in half the patients, and an increase in approximately one eighth. Kendrick, Balls and Rose (17) observed a decrease in the size of the gland in one fourth of their patients, no change in half of them, and an increase in the remainder. Taylor, Teitelbaum and Large (7) noted an increase in the size of the neck during methimazole therapy in 5 of 15 subjects, no change in 9, and a decrease in 1. In the

course of preoperative therapy with methimazole, Hallman and Bondy (12) found "marked involution" in the glands of 4 of 18 patients, in 3 of whom iodine had been an integral part of the treatment. In our own cases, the involution seen at the time of operation appeared in great measure to be due to the relatively large doses of iodine used for short periods. In the patients treated medically for long periods of time, we always added a "small" dose of Lugol's solution to the regimen—1 minim daily—as soon as the patient became euthyroid. This measure seemed to play an important role in the eventual involution and decrease in the size of the gland. However, the long-term study of Astwood and his associates (35) would lead us to believe that most patients will show involution of the gland during the prolonged administration of the antithyroid compound alone.

(d) *Incidence of toxicity.* Two of the patients with granulocytopenic reactions were first seen by our group when they entered the hospital following the onset of toxic symptoms. Statistically, therefore, they probably do not belong in the presently reported series of cases. However, we have included them in order to emphasize two things: (a) no effective antithyroid agent has yet been found which is entirely free of a toxic influence upon the bone marrow, and (b) suppression of bone marrow activity is most liable to occur in those patients who have received large doses of the drug over relatively long periods of time. Indeed, the latter point might well have been inferred from the early work of Williams, Kay and Jandorff, who determined the blood-tissue ratios attained following initial and subsequent doses of thiouracil (36).

Hines (37) states that in more than 1,200 cases of thyrotoxicosis treated with methimazole the incidence of granulocytopenia and agranulocytosis is approximately 0.18 per cent. If we omit the 2 cases coming under our observation merely to obtain hospital facilities for the care of their acute episodes, the incidence of such reactions in the present group of cases is 0.5 per cent. We agree with Bartels that the incidence of toxicity from methimazole therapy rises rapidly when the daily dose is increased beyond 40 milligrams (25). However, it is our impression that the reactions of the bone marrow to methimazole are somewhat milder than those reported for the thiouracils, and that none of them need be fatal if the drug is stopped when manifestations are first in evidence. The fact that such reactions occur at all should prompt the clinician to warn every patient to report the appearance of otherwise trivial symptoms.

SUMMARY

1. Methimazole (1-methyl-2-mercaptoimidazole) has been administered to 184 patients with hyperthyroidism in initial daily doses ranging from 15 to 60 milligrams and in maintenance doses of from 2.5 to 30 milligrams

for periods of time varying from fourteen days to four years. Lugol's solution was added to the regimen as the patients become euthyroid. "Large" doses of methimazole were used preoperatively, and "small" doses in connection with long-term medical care.

2. With adequate dosage, improvement was observed in three fourths of the patients within the first week of therapy.

3. Control of the thyrotoxic state required from two to twenty-six weeks, but 85 per cent of the subjects became euthyroid within the first five weeks. If patients are excluded in whom the initial doses were small, then 95 per cent of the remaining subjects became euthyroid within five weeks.

4. The progress of patients was followed by means of changes in weight, pulse rate, blood pressure, subjective symptoms, physical signs, basal metabolic rate and serum cholesterol level.

5. Of 90 patients in whom the size of the thyroid was serially followed during treatment, 12 showed an increase, 45 a decrease and 33 no appreciable change in the size of the gland.

6. In 37 patients, 35 with nodular and 2 with diffusely hyperplastic goiter, methimazole was used solely as a preoperative measure. In the remaining 147 cases, it was possible to obtain a satisfactory follow-up in 96. Of these 96 patients, 41 are still receiving methimazole; 42 recovered completely (but in 8 there was a recurrence); 7 were operated on; and 6 were given radioactive iodine.

7. Toxic manifestations, which appeared in 8 of the 184 patients, included pruritus, rashes, edema, urticaria, fever and granulocytopenia.

8. The highest percentage of "cures" was observed in patients below 35 years of age. In middle aged persons, *i.e.*, between 35 and 54 years of age, the thyrotoxicosis was brought under control less rapidly than in either the younger or the older individuals.

9. A reduction in the size of the thyroid was most consistently observed in the youngest of the three age groups.

10. Methimazole is a rapidly acting, relatively safe, effective antithyroid compound.

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Teaching Clinic

VAGARIES IN THE SYMPTOMATOLOGY OF CUSHING'S SYNDROME

IN 1932, Cushing published his monograph detailing 16 cases (11 females and 5 males) with a common symptomatology and established a syndrome characterized "in its full blown state by acute plethoric adiposity, by genital dystrophy, by osteoporosis, by vascular hypertension and so on. . . ."

It has been well established that the syndrome-complex known as Cushing's syndrome is attributable to hypercorticoidism. It is generally believed that the adrenal cortex elaborates many hormones. However, it should be mentioned that there are some who believe that the adrenal cortex elaborates but one or two precursor steroids and that the many steroids isolated from the adrenal cortex are either degradation products or are the result of the chemical methods employed in obtaining them. In either case, many of these steroids have been shown to possess certain biologic effects, whereas others appear to be physiologically inactive. In general, it may be said that there are three main categories into which these steroids fall:

(A) Anabolic (sex steroids)—such as androsterone and other substances with androgenic, estrogenic or progestogenic activity. Overproduction leads to virilization or defeminization in the female, and excess virilization or, at times, feminization of the male.

(B) Antianabolic (glucocorticoids)—typified by hydrocortisone and cortisone. Overproduction leads to hypergluconeogenesis, loss of protein, impaired carbohydrate tolerance, increased fat deposition, moon face, striae, osteoporosis, polycythemia and eosinopenia.

(C) Electrolytic (mineralocorticoids)—such as desoxycorticosterone. Excess production leads to retention of sodium, increased excretion of potassium, retention of water, and hypertension.

Hypercorticoidism suggests the overproduction of corticosteroid hormones. This may be due to hyperplasia, adenoma, or carcinoma of the adrenal cortex. When the corticosteroids produced by the adrenals cause signs and symptoms, the clinical picture will depend on which steroids are predominant. Frequently, the picture is well defined and points to the corticosexual group of corticosteroids, *i.e.*, androgen-like and gynecic-like hormones (adrenogenital syndrome). At other times it points to the corticometabolic group (Cushing's syndrome). Sometimes there is much overlapping and the clinical picture is not clear cut.

The corticometabolic syndrome, or Cushing's syndrome, occurs as a result of the overproduction of cortical hormone acting on carbohydrate, fat and protein metabolism. Most patients with this disturbance also show

changes in the sexual sphere, but it is thought that these features are the expression of a concomitant androgenic overactivity. This overactivity is usually such as to pseudomasculinize the patient, *i.e.*, the female is usually amenorrheic and hirsute, but without voice changes or enlargement of the clitoris. Frequently the male is, in a measure, demasculinized. At times the female patient with Cushing's syndrome is definitely virilized, and androgenic activity as measured by urinary 17-ketosteroid titers is considerably increased.

Regardless of whether the pathogenesis is primarily (a) a basophilic adenoma of the pituitary, (b) a lesion of the hypothalamus, or (c) an adrenal tumor or adrenal hyperplasia, hyperfunction of the adrenal cortex is necessary for the production of the signs and symptoms of Cushing's syndrome (except in the rare case of ovarian tumor of adrenal-rest origin).

Among the salient features of Cushing's syndrome are the following: (a) profound muscular weakness and fatiguability, (b) hypertension, (c) hirsutism with varying degrees of seborrhea and acne, (d) buffalo obesity, (e) amenorrhea in women, (f) impotence in men, (g) osteoporosis, (h) decreased resistance to infection, (i) purplish cutaneous striae, (j) easy bruisability, (k) plethoric moon facies, (l) occasionally nephrolithiasis.

Polycythemia, and mild leukocytosis with lymphopenia often occur. Marked depression of the circulating eosinophils (the count ranging from 100 down to 5 per cu. mm.) is a constant finding. Insulin resistance due to impaired carbohydrate utilization may be present. Some patients may exhibit mild degrees of psychosis. Hypopotassemia may accompany the alkalosis (increased serum sodium and carbon dioxide concentration) of Cushing's syndrome.

Too much reliance should not be placed on 17-ketosteroid excretion for a diagnosis of Cushing's syndrome. The values may be normal or elevated in adrenal hyperplasia and benign tumor, but are usually markedly elevated in malignant adrenal tumor. More important are the urinary corticoid values. Increased levels of urinary cortins are found as a rule if the urine is analyzed for formaldehydogenic corticosteroids or 17-hydroxycorticoids. However, though laboratory confirmation is most welcome in a suspected diagnosis of Cushing's syndrome, one must realize that such confirmation is not always present. Furthermore, it should be borne in mind that the vagaries of the signs and symptoms may be such as to baffle the physician further.

Many of the specific metabolic effects observed from prolonged administration of adrenocorticotropin and cortisone are similar to those seen in Cushing's syndrome. Large prolonged dosage of cortisone will induce increased gluconeogenesis with impaired carbohydrate utilization, negative nitrogen balance, polycythemia, neutrophilia, lymphopenia, eosinopenia, increased calcium and phosphorus excretion, a "Cushing-like" appearance, and even psychic changes. Prolonged adrenocorticotropin administration

may do as much and more, for adrenocorticotropin has been shown to stimulate the adrenal cortex to increase the secretion of urinary 17-ketosteroids and 17-hydroxycorticoids and other corticosteriods. Hirsutism and acne are observed occasionally after prolonged adrenocorticotropin therapy but rarely after cortisone therapy.

The following 2 cases are presented because each one differs from the other and both are atypical when compared with the classic text-book picture of the syndrome. The case reports that follow have not heretofore been published.

CASE I

The first case may be spoken of as a "pure" Cushing's syndrome, for it resembles closely the clinical picture occasionally seen after prolonged administration of cortisone. In the pure case the metabolic disorder should be so paramount and predominant as to overshadow or exclude disturbances in the sexual sphere.

Mrs. N. S. presented just such a case. This white female, aged 34 years, was admitted to the University Hospital on January 6, 1953, complaining of severe backache, gain in weight and amenorrhea. History revealed increasing weakness and obesity (gain of 40 pounds) over the previous four years.

Consultant's note: "This is the 'purest' case of Cushing's syndrome I've ever seen. Most cases are 'impure' and many are mixed 'adrenogenital and Cushing's.' Pure Cushing's syndrome should show disturbances associated with hypergluconeogenesis, *i.e.*, with the 'cortin' fraction, without any, or with very little, disturbance along androgenic and electrolytic lines. This patient shows only the slightest growth of hair on her upper lip and no hirsutism or hypertrichosis whatsoever elsewhere (Fig. 1a). There is no acne, no marked obesity. The moon face and slight protuberance of the abdomen are the only evidence of slight obesity. The extremities are normal (Fig. 2a). The skin is thin, the complexion is plethoric, and there are well developed purplish striae on the abdomen and thighs. There is loss of libido and marked general weakness. X-ray studies reveal marked osteoporosis with collapse of the vertebrae (Fig. 3). This accounts for the accentuated buffalo hump and the severe back pain. The blood pressure is only slightly elevated to 140/90. There are no voice changes and no enlargement of the clitoris. Her glucose tolerance test shows mild decrease in carbohydrate tolerance. There is no insulin resistance on performing the insulin tolerance test. Her fasting eosinophil count is much depressed (37 per cu.mm.). She has no polycythemia. Her vaginal smear is hypoestrogenic. Her 17-ketosteroids should be normal, but her cortins should be elevated. *Impression:* Cushing's syndrome."

On further studies, the levels of serum sodium, potassium, and carbon dioxide combining power were within the limits of normal. The serum level of nonprotein nitrogen was 38 mg. per 100 ml. and 1 plus albumin was found in her urine specimen. The Thorn test showed a drop in the eosinophil count from 31 to 25 in four hours. Presacral insufflation of air revealed an enlarged right adrenal gland. Urinary 17-ketosteroids were in the low normal range and the values for three different determinations were 7.1, 4.0 and 6.3 mg. per twenty-four hours (our normal range for females is from 7 to 13 mg.).[1] The formaldehydogenic steroids appeared to be within normal range—0.17, 0.6 and 0.48 mg. per

[1] 17-Ketosteroid assays were performed according to the method of Pincus, G.: *J. Clin. Endocrinol.* **5**: 291, 1945.

FIG. 1a. *Case 1.* Cushing's syndrome. Note plethoric "moon" face and absence of hirsutism.

FIG. 1b. *Case 2.* Mixed adrenogenital-Cushing's syndrome. Note the plethoric moon face, extreme hirsutism and acne.

twenty-four hours (our normal values range from 0.1 to 0.8 mg.)[2]. However, the creatinine excretion also was very low—676, 584 and 591 mg. per twenty-four hours (normal values 1,000 to 1,500 mg.).

Operation was performed on January 23, 1953, after preparation of the patient with cortisone and adrenocorticotropin. The tumor was removed and the histopathologic report was adenoma of the adrenal gland. Medication was continued in descending doses for several days. The patient made an uneventful recovery (Fig. 2b). Figure 4 traces her progress before operation and for a period of ten months after operation, showing blood pressure values, fasting eosinophil counts, and levels of 17-ketosteroids, formaldehydogenic steroids, and urinary creatinine.

CASE 2

The second case may be spoken of as a mixed adrenogenital-Cushing's syndrome. The virilization is so striking as to overshadow the underlying metabolic disorders. The classic picture of Cushing's syndrome is one of defeminization rather than masculinization. Here we have a patient who was markedly masculinized, yet had all the earmarks of Cushing's syndrome.

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[2] Cortin (corticoid) determinations were made by the formaldehyde reducing method described by: Daughaday, W. H., Jaffe, H., and Williams, R. H.: *J. Clin. Endocrinol.* **8**: 166, 1948.

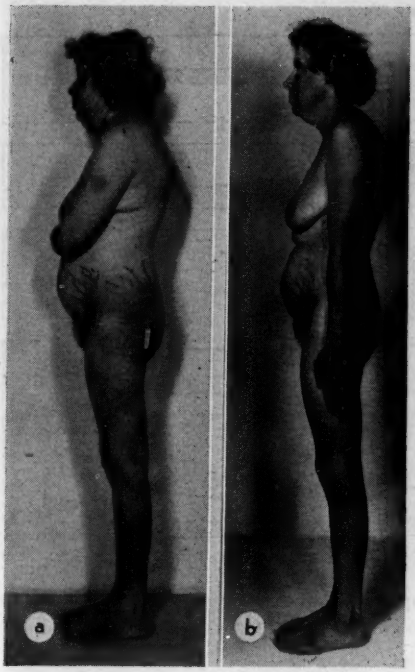

FIG. 2a. *Case 1.* Cushing's syndrome. Note the bruisability of skin, forearm, and hip; the kyphosis (buffalo hump); the obesity confined to the face and abdomen sparing the extremities; and the abdominal striae.

FIG. 3. *Case 1.* Cushing's syndrome. Note marked osteoporosis and collapse of several of the thoracic vertebrae.

FIG. 2b. *Case 1.* Ten months after surgical removal of adrenal adenoma. Note fading of striae, and persistence of kyphosis due to collapsed vertebrae.

Mrs. D.A., a 23-year-old white female, was first seen on July 26, 1952. She complained of amenorrhea of six months' duration, deepening pitch of the voice of two months' duration, marked hirsutism, and acne (Fig. 1b). She had had an attack of kidney colic in January 1952. Her face was full, round and plethoric. She was muscular and strong, with purplish striae on the abdomen and thighs (Fig. 5a). The clitoris was considerably enlarged (3+). Her sex desire had increased. Her blood pressure was 160–180/100. She had a polycythemia of 6,490,000 red blood cells and a hemoglobin of 18 grams per 100 ml. Her fasting eosinophil count was depressed, *i.e.*, 25 and 44 per cu. mm. The Thorn test showed a fall in eosinophils from 43 to 31 per cu. mm. in four hours. The results of glucose tolerance and insulin tolerance tests were within limits of normal. The vaginal smear was mildly hypoestrogenic. Her urinary 17-ketosteroids were 145.15 mg. per twenty-four hours (normal values 7 to 13 mg.). The pregnanediol excretion was 27.52 mg. per twenty-four hours (normal 0.3 to 3 mg.). The formaldehydogenic steroids were 2.71 mg. per twenty-four hours (normal 0.1 to 0.8 mg.). The Allen test (for the steroid thought to be dehydroisoandrosterone) gave a positive reaction.

X-ray studies: An intravenous pyelogram revealed that the left kidney was moderately depressed by a mass in the suprarenal area which measured about 10×10 cm. (Fig. 6). The sella turcica was normal. There was no osteoporosis.

A diagnosis of malignant adrenal tumor was made because of the x-ray evidence in

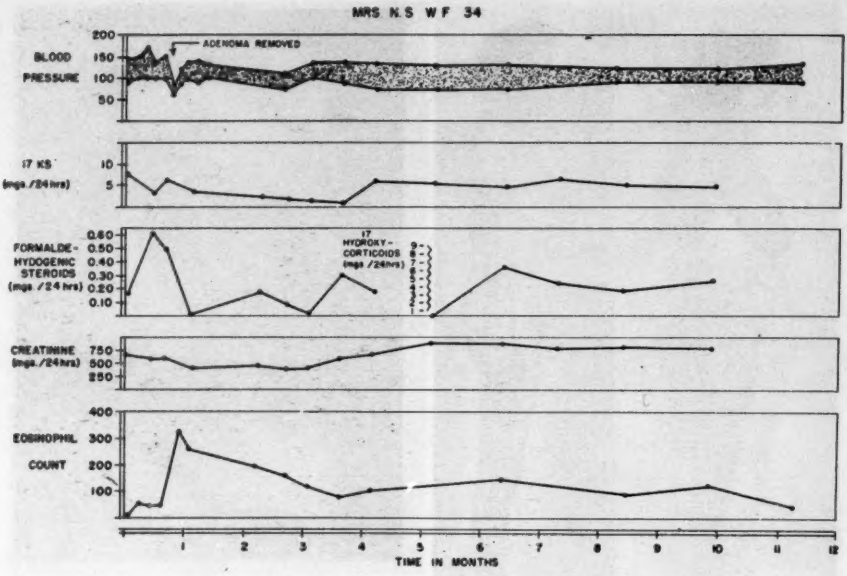

FIG. 4. Hormonal assays, fasting eosinophil levels, urinary creatinine values and blood pressure readings during period of observation in *Case 1*—"pure" Cushing's syndrome.

the presence of positive results with the Allen test. Operation was performed on August 6, 1952. The adrenal tumor was adherent to the left kidney, and both were removed. The histopathologic report was solid carcinoma of the adrenal gland. The patient was prepared preoperatively with cortisone and adrenocorticotropin and was given these preparations postoperatively in descending doses.

One and one-half years after the operation the patient is well (Fig. 5b). Blood pressure, hormone assays, and eosinophil counts have returned to normal levels and are recorded for this period in Figure 7. Allen test results remain negative. Menses have become cyclic, the acne and hirsutism have disappeared except for a few hairs which remain on the upper lip, and the pitch of the voice is higher, though still lower than normal.

DISCUSSION

In the consideration of the 2 cases reported here, several pertinent questions arise.

Question. Why did one patient have marked osteoporosis, whereas the other had none?

Answer. Osteoporosis in Cushing's syndrome is an expression of hypergluconeogenesis. It is part of the catabolic or rather, antianabolic, process. When the patient is in negative protein balance, osteoporosis and collapse of the vertebrae will occur because of loss of bony protein matrix, coincident with which there is loss of calcium. Excess production of cortisone-like hormones by the adrenal or the prolonged administration of such hormones will induce osteoporosis. In cases in which there is concomitant or compensatory overproduction of anabolic hormones such as androgens,

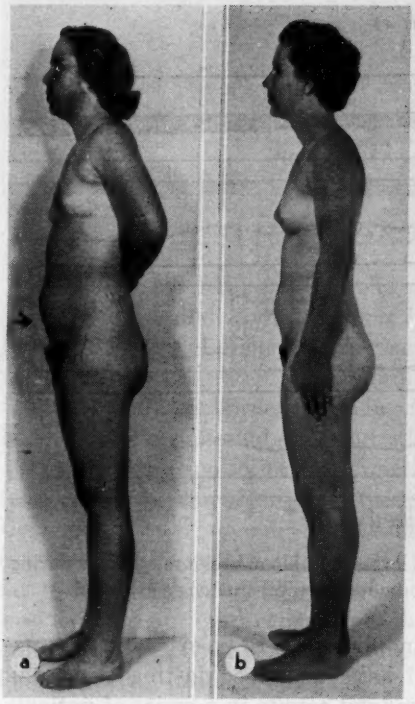

FIG. 5a. *Case 2.* Mixed adrenogenital-Cushing's syndrome. Note masculine body build, hairiness of abdomen (arrow), and striae of abdomen and thighs.

FIG. 6. *Case 2.* Mixed adrenogenital-Cushing's syndrome. Intravenous pyelogram: note displacement of left kidney by large tumor (arrows).

FIG. 5b. *Case 2.* Six months after removal of adrenal carcinoma. Note improvement in body build, and in breast development.

the catabolic process is modified and negative protein balance may not occur. Hence, in markedly hirsute and virilized patients with Cushing's syndrome, osteoporosis may not be present.

Question. Why do these 2 patients with Cushing's syndrome show such variations in the degree of hirsutism?

Answer. It must be recalled that Cushing's syndrome is primarily a corticometabolic disorder. Whether there is conversion of some of the excess glucocorticoids into 17-ketosteroids with androgenic activity, or whether there is a concomitant elaboration of androgenic steroids, is difficult to say at the present time. Hirsutism is an expression of androgenic activity and the degree of hirsutism will depend on several factors: (a) the end-organ responsiveness to the androgens, and (b) the level and duration of androgenic activity. Thus the hair-follicle apparatus in some patients is readily stimulated by androgens, but in others it is not.

Question. What is the significance and diagnostic value of the fasting eosinophil count in Cushing's syndrome?

Answer. Since the administration of corticosteroids such as cortisone

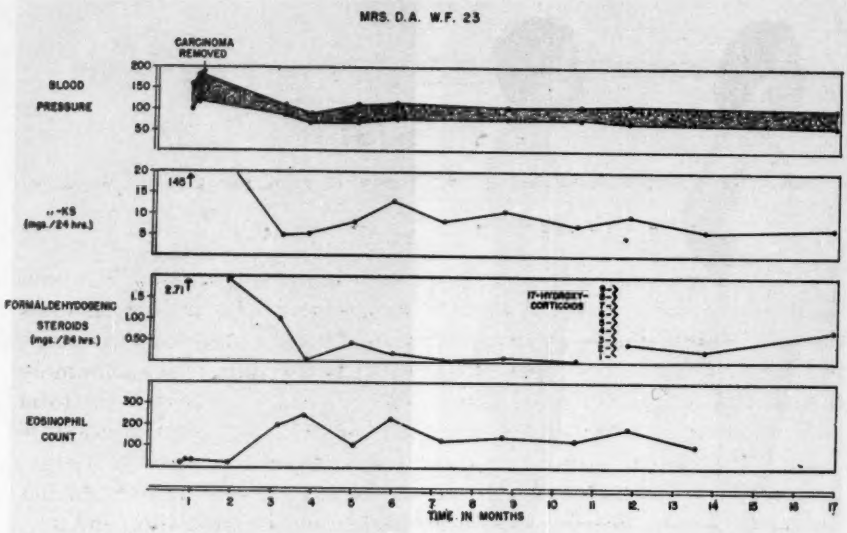

FIG. 7. Hormonal assays, fasting eosinophil levels, and blood pressure readings during period of observation in *Case 2*—mixed adrenogenital-Cushing's syndrome.

will lower the level of circulating eosinophils, it is to be expected that patients with Cushing's syndrome will have depressed fasting eosinophil counts. In comparing the fasting eosinophil counts in a series of patients with Cushing's syndrome and in patients with primary adrenogenital syndrome, it was found that in the former the counts were always much below 100, whereas in the latter they were normal (100–300) or above normal. It may be said, therefore, that a patient with a high or normal eosinophil count probably does not have Cushing's syndrome. The corollary, however, does not necessarily follow, to wit, that a low circulating eosinophil count is indicative of excess glucocorticoid activity.

CONCLUSIONS

The wide spectrum in the signs and symptoms found in Cushing's syndrome is stressed. Two patients with proved Cushing's syndrome are presented, whose signs and symptoms placed them at either end of this spectrum. One patient with so-called "pure" Cushing's syndrome was very weak, markedly osteoporotic and free from hirsutism, but the other patient, a case of mixed adrenogenital-Cushing's syndrome, was strong, virilized, and free from osteoporosis.

ROBERT B. GREENBLATT, M.D.

Department of Endocrinology,
Medical College of Georgia,
Augusta, Georgia

Editorial

ESTIMATION OF BODY FAT

FAT comprises the largest solid moiety of the human body. The well nourished adult contains about 11 kilograms of cellular solids and less that 5 kilograms of minerals, whereas his total body fat ordinarily amounts to 12 or more kilograms. Other than water, the amount of fat varies more than any body constituent, ranging from less than 2 per cent of the total body weight in profound undernutrition, to more than 40 per cent in severe obesity. In addition to its vital function as the main storehouse of energy, fat contributes short-chain intermediates for the synthesis of steroids, has the capacity to remove fat-soluble materials from the circulation and may possess other important attributes (1). The apparent relationship of excess fat to hypertension, cardiac disease, diabetes, degenerative vascular disease and perhaps even cancer, is of great importance and most authorities harbor the opinion that the obese person leads neither a happy life nor a long one. The problems incident to obesity are of great professional interest to most physicians and of personal interest to many. Despite these facts, even gross measurement of the fatty phase of the body has been sorely neglected. This discrepancy in our knowledge, which looms great in contrast to the more detailed knowledge of other body constituents and compartments, undoubtedly stems from lack of methods for the measurement of body fat.

During the past few years several technics have been developed which permit the *in vivo* analysis of the human body and which show promise in the quantitative estimation of the total body fat. In a recent broad review of the subject, Keys and Brozek (2) have outlined four methods whereby the relative degree of leanness-fatness may be ascertained: 1) determination of body density, 2) measurement of total body water, 3) application of anthropometric measurement (especially that of skin folds), and 4) use of fat-soluble indicators. The last method still lacks sufficient refinement to allow clinical application, and skin-fold measurement is too gross for precise analysis. This discussion is consequently concerned mainly with estimation of body fat by measurement of body specific gravity. A brief discussion of total body water measurement is included because of the basic similarity of this method to densitometric technics.

Although measurements of body specific gravity had been made previously, not until 1942 was this type of analysis conducted with sufficient precision by Behnke and his co-workers, to permit clinical application (3). From his studies in normal subjects, Behnke secured data to indicate that the comparatively low specific gravity of fat renders this substance the main determinant of corporeal density. Thus it seemed reasonable that measurement of the specific gravity of the human subject would permit estimation of his total body fat. Rathbun and Pace measured the specific gravity of guinea pigs and demonstrated by carcass analysis the validity of Behnke's concept (4). Data secured from these studies were utilized for the construction of a fat prediction equation based on the measurement of specific gravity, *i.e.*

$$\% \text{ Fat} = 100\left(\frac{5.548}{\text{sp. gr}} - 5.044\right).$$

The essential measurement in this technic is that of body volume. Based on Archimedes' principle, body volume is determined by hydrostatic weighing, *i.e.*—equivalent volume = weight in air minus weight in water. Body specific gravity is obtained from the ratio of the body weight to that of equivalent volume. Weight in water is secured by suspending the subject beneath the surface of the water by a line attached to a sensitive spring scale, a lead belt being used to overcome buoyancy. Weights are obtained during both full expiration and complete inspiration; the difference between these values, which should check closely with measured vital capacity, furnishes assurance that on successive determinations the patient is expiring to constant volume. True submersed weight is obtained by correction for residual lung volume and weight of the belt. Strictest accuracy in densitometric determination by the specific gravity method is obtained by correction for the temperature of the water, although in following changes in one subject it is necessary to maintain only relatively constant temperature.

On the basis of studies by Behnke, Keys and their associates, it would appear that densitometric estimation of fat is not only feasible and reproducible, but also fairly accurate in the normal human subject. Pathologic loss or accumulation of fluid, with consequent distortion of the normal relationships between body fat and total body water, renders tenuous any precise attempt at body dissection. Keys has attempted to circumvent hydration abnormality by correcting for abnormal change in body weight (assumed to involve primarily the extracellular fluid space) by determination of thiocyanate space. Such correction is based on the assumption that the volume of distribution of thiocyanate exceeds the true extracellular

fluid space by a constant percentage. Such a supposition is open to question, however, for it seems very probable that such pathologic states as would tend to expand the true space would allow an even greater increase in the volume of distribution of thiocyanate. Furthermore there is evidence that abnormal accumulation of fluid involves both the cellular and extracellular compartments (5). It is possible, however, that a method for determination of total body water (D_2O or antipyrine space) which is less susceptible to distortion, may be applicable for the purpose of correcting hydration abnormalities.

The original observations of Behnke, as well as the confirmatory studies on smaller animals, indicate that fat and water vary inversely. Consequently, not only can total body water be estimated from body specific gravity, but direct measurement of total body water will permit estimation of the size of the lean body mass and consequently, with the assumption of a constant mineral fraction, the total body fat. Whereas determination of body fat by specific gravity is based on the assumption that there exists a lean body mass, of specific gravity 1.099, the use of total body-water measurement to reach the same end assumes that the lean body mass is 72 or 73 per cent water. These two assumptions, both based on measurements of Rathbun and Pace, are closely related and application of total body water for estimation of body fat, though simple, is not truly different from the method outlined above. Although it is readily applicable to the sick patient, because of the error resulting from even a slight hydration abnormality the total body-water method cannot be used for accurate measurement of fat in starvation, severe obesity and for that matter, in the majority of disease states.

A somewhat similar approach was made by McCance and Widdowson, who estimated the size of the "fat-free body" based on measurement of both total body water (urea space) and extracellular fluid (thiocyanate space) (6). The difference between the two is considered to be the intracellular space. Further calculation is dependent upon the vigorous assumption that water comprises 67 per cent of the weight of cells.

All of these allied methods of body dissection possess inherent weaknesses, all require certain assumptions which are difficult to prove and all are readily applicable only in the normally hydrated individual. Measurement of total body water has the major advantage of simplicity but is less accurate and is of extremely dubious value in the sick patient. Densitometric analysis is more precise, allows correction for abnormal hydration but is difficult to conduct in the ill subject—impossible in the bedridden patient. In this laboratory, specific gravity measurements have been demonstrated as both feasible and reproducible in patients with myxedema,

Cushing's syndrome, chronic skin disease, scleroderma, leukemia, rheumatoid arthritis and severe obesity (7). Other densitometric methods may allow analysis even of the extremely ill patient. Volume displacement of air has been refined by Wedgewood but as yet with insufficient precision to allow accurate measurement of fat (8). The helium concentration technic of Siri shows considerable promise (9). Even with further refinement of these simpler technics, or fulfillment of current hopes of utilization of fat-soluble indicators, density measurement will still be needed as a standard of reference for these and subsequent methodological advances.

Applications of densitometric measurements and related technics, have already made substantial contribution to our knowledge of metabolism. Welham and Behnke demonstrated in a group of professional football players that although a number of these subjects were by conventional standards ineligible for either life insurance or military service on the basis of their "obesity," analysis by specific-gravity measurement indicated the amount of body fat to be expected in the well conditioned athlete (10). In other words, they were not fat but large. Behnke and his associates have also drawn attention to the fact that the "lean body mass" is a far more suitable reference for numerous physiologic processes, and organ size than are the conventional measurements (11). This appears to be true of the metabolic rate and has been documented with respect to cardiac output (12). Undoubtedly, a similar correlation exists with many other vital functions. Moore and his associates have studied changes in body fat, obtained indirectly by measurement of D_2O space and in some instances checked with nitrogen balance, which indicate that surgically traumatized individuals utilize body fat at a much greater rate than had been previously suspected (13). Behnke and his associates have studied changes that occurred with loss or gain in weight, which indicate that the specific gravity of adipose tissue is approximately .940; similar results have been obtained in studies of depletion and repletion by Keys and his group. Studies in this laboratory have indicated the feasibility of utilizing the specific-gravity method for quantitation of body fat in patients with metabolic disease, as well as measurement of changes in fat which result from various hormonal influences (14). In terms of metabolic change it must be emphasized however, that the newer technics of body dissection supplement, but do not replace, older methods of study. Thus densitometric analysis will offer most, if combined with other technics such as nitrogen or water balance or measurement of total body water and extracellular fluid space. This procedure provides not only additional information relative to change in fat but serves as a check on the other methods of measurement. Gross and cumbersome though it may be, densitometric measurement of body composition

offers a method whereby quantitative analysis can be made of a substance —fat—which hitherto has eluded adequate measurement.

LAURENCE H. KYLE, M.D.

Department of Medicine,
Georgetown University Hospital,
Washington 7, D.C.

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Questions and Answers

(Questions submitted at the 1953 Postgraduate Assembly of the Endocrine Society and the University of Minnesota)

Answers by Randall G. Sprague, Mayo Clinic, Rochester, Minn.

QUESTION: What is the treatment of idiopathic hypoglycemia in adults?

ANSWER: The answer to this question depends upon what is meant by the term "idiopathic hypoglycemia." Most frequently, the term is applied to individuals, usually women, in whom mild symptoms of hypoglycemia, associated with moderate depression of the level of the blood sugar, occur two to three hours after a meal. The hypoglycemia in such cases probably represents an exaggeration of the drop in the blood sugar which normally occurs two to three hours after the ingestion of glucose or a high carbohydrate meal. There is no convincing evidence that it represents overproduction of insulin or any other disturbance of endocrine function. In such individuals the blood sugar is well maintained during fasting, in contrast to the fall in blood sugar which occurs during fasting in patients with "organic hypoglycemia" due to islet-cell tumor, anterior pituitary insufficiency, adrenocortical insufficiency, or extensive destructive lesions of the liver.

Since the condition is frequently associated with other symptoms attributable to nervous disturbances, it is important that it be established beyond doubt that the symptoms of "hypoglycemia" are actually related to significant depression of the level of the blood sugar, and are promptly relieved by the ingestion of sugar. If such a relationship can be established, treatment consists of a high-protein diet, or intermeal feedings, or both.

QUESTION: What is the cause of night cramp in diabetics?

ANSWER: The cause of muscular cramps at night, usually in the legs, in diabetic patients is not known with certainty. Such cramps are not a symptom of arterial disease of any kind. Moss and Herrmann (1) suggested that they may result from stimulation of the muscle by unknown metabolites which accumulate as a result of venous stasis. The observation of some patients that cramps are likely to occur during a period of rest following unusual muscular activity lends some support to this theory. Whether or not there is an abnormality of muscle metabolism in diabetes which favors the development of cramps is not known.

The same symptom occurs in patients without diabetes and is presumably on the same, or a similar, basis. Moss and Herrmann (1) noted that about half of a group of 20 patients with night cramps had diabetes. The presence of pathologic conditions in the veins was the next most frequent finding, supporting the concept that venous stasis may play a role. Cramps also occur commonly in pregnancy, again suggesting that venous stasis may be a factor in their production.

Small doses of quinine sulphate (5 grains at bedtime) sometimes seem to prevent the occurrence of cramps, presumably through a direct action on muscle or on the motor end-plates.

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Answers by E. C. Reifenstein, Jr., Schering Corporation, Bloomfield, N. J.

QUESTION: How long must a postmenopausal woman be treated with steroids before x-ray examination shows an increase in bone density?

ANSWER: A definite increase in bone density that will be detectable to all who compare the x-ray films does not occur in most patients until they have been treated for from eight to ten years with steroids. Clinical benefit, particularly loss of bone pain, is apparent, however, within weeks or months in most cases.

QUESTION: What are the advantages of giving androgen with estrogen in postmenopausal osteoporosis?

ANSWER: The combination of androgen and estrogen has these advantages in postmenopausal osteoporosis:

1. More retention of calcium and phosphorus in the proportions that exist in bone than can be obtained with either type of steroid alone.
2. Less effect of estrogen on the breast (engorgement) and on the uterus (withdrawal bleeding).
3. Less effect of androgen in producing masculinizing symptoms (hirsutism, acne, and hoarseness)

The estrogen should always be given intermittently; the androgen may be given on the same basis, or may be given continuously.

QUESTION: Will administration of estrogens and androgens aid in the healing of fractures in postmenopausal women?

ANSWER: It is the impression that they will, although such a claim is hard to prove by clinical studies. Certainly, the steroids will do no harm, and since they may help, they should be tried in such cases.

QUESTION: What is the place of strontium therapy in osteoporosis?

ANSWER: Dr. E. Shorr and his associates have reported that strontium can be deposited in the bones of patients with osteoporosis by steroid therapy, when such patients will no longer retain calcium. This work is encouraging; it needs confirmation in another laboratory.

QUESTION: Would you use gonadal steroid therapy in a woman with clinical osteoporosis who has had a uterine cancer apparently successfully removed five years previously?

ANSWER: The amount of disability derived from the clinical osteoporosis is the determining factor in deciding whether or not to treat the patient with steroids. Therapy should not be withheld because the patient has had a uterine cancer removed, apparently completely, five years before; but she should be watched more carefully because of this history.

The Endocrine Society

THE 1955 ANNUAL MEETING

The Thirty-seventh Annual Meeting of The Endocrine Society will be held in the Chalfonte-Haddon Hall Hotel, Atlantic City, New Jersey, on Thursday, Friday and Saturday, June 2, 3, 4, 1955.

Dr. Matthew Molitch, 705 Pacific Avenue, Atlantic City, New Jersey, is in charge of the local arrangements for the meeting.

All Scientific Sessions will be held in the Chalfonte-Haddon Hall Hotel. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The annual dinner is scheduled for Friday, June 3, at 7:30 p.m., preceded by cocktails at 6:30 p.m.

All members are urged to make hotel reservations immediately, as the hotels expect to be filled to capacity. Correspond directly with Chalfonte-Haddon Hall, advising time of arrival and departure. Make your reservations now and avoid disappointment.

Those wishing to present papers, (time limited to ten minutes) should send four copies of the title and abstract to the Vice-President, Dr. E. B. Astwood, 30 Bennet Street, Boston 11, Massachusetts, not later than *February 1, 1955*. It is imperative that the abstracts be informative and complete with results and conclusions in order that they may be of reference value and suitable for printing in the program and Journals of the Society. The following regulations for the preparation of abstracts and titles must be carefully followed to insure consideration of the paper for the program:

1. Abstracts may not exceed two hundred words, or equivalent space, exclusive of title. Neither footnotes nor acknowledgments to sponsors can be published. References, if used, should be placed in the body of the text. The abstract should consist of a single paragraph, if possible. Structural chemical formulas cannot be used.
2. The title heading should be arranged as follows:
   - Line 1. Title, not to exceed fifteen words.
   - Line 2. Author/s. The name of each nonmember-author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of nonmembers who are introduced, *i.e.*, who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be given after his name.
   - Line 3. Institute of origin and city in which institution is located.

3. The body of the abstract, typed double-space, should follow the heading. The original copy should be on bond paper. There should be three copies.
4. Abstracts should be letter-perfect, as there will be no opportunity for proof reading by authors.
5. Abstracts not conforming to the foregoing regulations will be returned to the authors.

RECIPIENT OF 1954 CIBA AWARD

The Ciba Award of The Endocrine Society, established to recognize the meritorious accomplishment of an investigator, not more than 35 years of age, in the field of endocrinology has been awarded to Dr. Isador Nathan Rosenberg of Boston.

If anyone can qualify for the distinction of being a Bostonian, Dr. Rosenberg can. He was born in Boston on May 19, 1919, and attended the Boston Latin School, from which he graduated in 1936. He received the Bachelor of Arts degree with honors from Harvard College in 1940, and the M.D. degree from Harvard Medical School in 1943, magna cum laude and at the head of his class. Had it not been for the war he would have been in Boston uninterruptedly ever since, but he spent two years abroad as a Captain in the Medical Corps of the Army in the Pacific, as soon as he had finished his internship in the Boston City Hospital. He returned to the Biochemical Department at Harvard before resuming his clinical training at the Boston City Hospital, where he spent a total of three years, completing his residency in 1949. Since 1949 Dr. Rosenberg has been engaged in research, clinical work, and teaching at the New England Center Hospital and Tufts College Medical School, at first as a Postdoctoral Research Fellow of the U. S. Public Health Service for two years, then as a Research Associate in the Hospital and Instructor of Medicine at Tufts.

Dr. Rosenberg's research activities began while he was still an undergraduate at Harvard College, under the guidance of Dr. Joseph Aub and his associates, Drs. I. T. Nathanson, R. W. Rawson, and Waldo Cohn. Here he completed a study on "Phosphorylation in Tumors," which involved the use of radioactive phosphorus; this served as his honors thesis. During his medical school days and again after returning from the Philippines, he worked with Professor A. Baird Hastings and his associates and made fundamental contributions on the incorporation of carbon dioxide into glycogen and on the metabolism of carbohydrate precursors using radioactive carbon. Most of his more recent independent investigations in his current post have been concerned with the thyroid and pituitary glands. He developed new chromatographic methods for the separation and isolation of the iodine-containing compounds of the thyroid gland, and for this

work he was awarded the Van Meter Prize of the American Goiter Association three years ago. Chromatographic methods were also applied to the problem of the nature of the thyroid hormone in Graves' disease. Dr. Rosenberg showed that the hormone is thyroxine, thus dispelling the older idea of some mysterious poisonous secretion from the thyroid gland in thyrotoxicosis. The mechanism of action of antithyroid drugs was clarified by the simultaneous and independent discovery by Dr. Rosenberg and by Arnott and Doniach that all classes of antithyroid compounds interfere with peroxidase action, strongly supporting the theory that the first step in thyroid hormone synthesis is an oxidation brought about by a peroxidase-like enzyme in the gland.

In the pituitary field Dr. Rosenberg was first associated with a very extensive clinical trial of corticotropin during the early years of such therapy. He furthered the development of more effective forms of corticotropin and of longer acting forms for clinical use, and was the first to show that oxycellulose is a good absorbing medium for the hormone. He later showed that the purified corticotropic preparation exerted potent metabolic effects. The formerly elusive factor or factors variously described as fat-mobilizing factor or adipokinin, and the specific metabolic principle (promoting the mobilization and oxidation of depot fat with a consequent increase in oxygen consumption, decrease in respiratory quotient and ketosis) were accounted for by the pituitary fraction containing corticotropin, and were shown not to be specific effects of the pituitary growth hormone.

Dr. Rosenberg was one of the first to apply the technic of electrophoresis on paper to the study of lipoproteins and was able to show that the incidence of atherosclerosis bore no relationship to the pattern of lipoproteins in the serum. A similar technic was profitably applied to the analysis of the components of pituitary hormone preparations.

When not engaged in his research activities, Dr. Rosenberg is said to engage himself in a number of quiet pursuits. He has a reputation as an angler, music critic, and football fan, and frequently delights his friends with a well-turned phrase. He is said to be a bachelor, but there is a recent rumor that this statement may soon be subject to correction.

The American Goiter Association

THE 1955 ANNUAL MEETING

The next annual meeting of the American Goiter Association will be held in Oklahoma City, Oklahoma, at the Skirvin Hotel, April 28, 29 and 30, 1955.